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INTERORGANISMIC TRANSFER OF A DISCRIMINATED OPERANT

by

PERRY STUDLEY KINKAIDE

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF PSYCHOLOGY

EDMONTON, ALBERTA

APRIL, 1967

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Interorganismic Transfer of a Discriminated Operant" submitted by Perry Studley Kinkaide in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

Several investigations during the last two years have reported specific behavioral alterations in rats as a function of their being injected with a brain extract prepared from trained rats. The behavioral alterations are in correspondence with the training of the donor Ss. The research described in this thesis attempted to demonstrate in a similar manner an interorganismic information transfer from trained rats (donors) to untrained rats (recipients). The behavioral task was a modified S^D-S^Δ operant discrimination schedule described by Carlton (1959).

Two preliminary investigations established that an S's responding at asymptote on the S^D-S^Δ schedule was primarily under stimulus control. It was also found that performance was more stable and learning more rapid when visual rather than auditory discriminative stimuli were employed.

Three treatment conditions were employed for testing the specific transfer. The recipients were injected IP with a brain extract prepared from individual donors. The extract contained both protein and RNA. One recipient group was tested with the same stimulus conditions as their donors (Positive prediction) while a second recipient group was tested with the opposite stimulus conditions (Negative prediction). A third recipient group received a brain

ABSTRACT

Several investigations during the last two years have reported specific behavioral alterations in rats exposed as a function of short period interval with a brain extract prepared from trained rats. The behavioral alterations are in correspondence with the training of the donor rats. The research described in this present attempted to demonstrate a similar manner an interorganismic information extract from trained rats (donors) to untrained rats (recipients). The behavioral task was a modified S_D-S_Δ operant discrimination schedule described by Carlton (1956). Two preliminary investigations established that an $\overline{S}$'s responding at asymptote on the S_D-S_Δ schedule was

based (positive transfer) while a second recipient group received a brain extract contained both brain and RNA. One recipient group trained with the same stimulus conditions as donors but with a brain extract prepared from inadequate donors. The recipients were injected 1½ hrs before training.

Three treatment conditions were employed for testing each recipient group.

Several investigations during the last two years have reported specific behavioral alterations in rats exposed as a function of short period interval with a brain extract prepared from trained rats. The behavioral alterations are in correspondence with the training of the donor $\overline{S}$s. The research described in this present attempted to demonstrate a similar manner an interorganismic information extract from trained rats (donors) to untrained rats (recipients). The behavioral task was a modified S_D-S_Δ operant discrimination schedule described by Carlton (1956). Two preliminary investigations established that an $\overline{S}$'s responding at asymptote on the S_D-S_Δ schedule was

extract from untrained Ss and was tested in a similar fashion as the Positive and Negative recipient Ss.

During S^{Δ} it was found that over the four days of testing the Positive recipient group's performance reflected its donor group's performance in the presence of S^{Δ}. On the other hand, the Negative recipient group's performance corresponded with its donor group's performance in the presence of S^{D}. As well, the recipient Ss' performances (response frequency and response latency to S^{D}-onset) were correlated with their respective donors.

It was concluded that an information transfer had been effectively demonstrated and that the information was specific to the performance of the donor. On the basis of these results it was further suggested that the memory state is chemical in nature.

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Introduction

Events in the environment of an organism can produce changes in the nervous system which subsequently influence behavior. The altered state of the nervous system is referred to as memory while the process(es) of modification is known as learning. Recently a number of interdisciplinary studies within psychology and biochemistry have been performed which suggest that learning results in biochemical changes in the nervous system, perhaps involving changes in the structure of large organic molecules. These studies have demonstrated an apparent "information transfer" from one organism to another via the injection of an extract of the brain of a trained rat (donor) into an untrained rat (recipient). A successful demonstration of a "learning savings" by the recipient has been heralded as evidence supporting a molecular interpretation of memory. The molecular hypothesis was originally put forth by Katz and Halstead (1950) and Halstead (1951). They proposed that external stimulation of an organism results in the transformation of nucleoproteins from random to oriented, organized configurations. The reorganized nucleoproteins then act as templates upon which replica proteins are formed. Ultimately the reorganized protein replicas reside in the neural membrane where they subsequently influence neural activity.

All attempts to demonstrate a "transfer" of learned responses interorganismically have employed a brain-extraction-injection technique. The technique involves four steps: (1) The training of a donor, (2) The preparation of an extract from the donor's brain, (3) The injection of the extract into a recipient, and (4) The testing of the recipient on the same task to which the donor had been trained. The underlying hypothesis is that the memory state is molecular in nature, and that following the injection of the "trained-brain extract" into an organism, the active encoded molecular substance(s) becomes potentially functional in processes which ultimately direct the recipient's behavior.

The "startle response"served as a behavioral index in the first reported "memory transfer" experiment in vertebrates. Ungar and Oceguera-Navarro (1965) habituated the startle response of seven rats to an auditory stimulus. Following habituation, a pooled homogenate of the rats' brains was prepared. The extract was then injected intraperitoneally (IP) into albino mice. A control group was injected with a similarly prepared homogenate from non-habituated rats. Results indicated that the mice which were recipients of the sound-habituated rat brain habituated more rapidly than those subjects (Ss) given normal brain. The initial response frequency was sharply reduced in the experimental mice.

A subsequent study by Ungar (1966) enabled Ungar to conclude that suppression of the startle response was not due to a general depressant action of the brain extracts. Extracts from sound-habituated rats were injected into naive mice. The mice demonstrated a facilitated habituation to sound but not to an air-puff. As well, Ss which received extracts from air-puff habituated animals rapidly habituated to an air-puff but not to sound. This evidence is indicative of the transfer of specific information which Ungar proposed is recorded in chemical form.

Babich, Jacobson, Bubash, and Jacobson (1965) also reported a highly significant "memory transfer". This experiment has become the standard, or at least the most frequently attempted, "transfer" experiment. After training several rats to approach a food cup at the sound of a click, a pooled brain extract was prepared which presumably isolated ribonucleic acid (RNA) exclusively from the brains of the trained rats. A phenol extraction technique was used. The extract was injected IP into 24-hr. food deprived rats which were untrained but click-adapted. Five testing sessions were subsequently given at 4, 6, 8, 22, and 24 hrs. after injection. The recipients manifested a significant ($P<.002$, one-tailed test) tendency as compared with controls to approach the food cup when the click, unaccompanied by food, was presented. The controls consisted of Ss injected with a

brain extract prepared from naive Ss. The results, the authors conclude, suggest that RNA was probably the active transfer factor.

Jacobson, Babich, Bubash, and Jacobson (1965), like Ungar (1966), ventured to determine the degree of stimulus specificity of the "transfer phenomenon". Rats were trained to approach a food cup when either a click (Group 1) or a blinking light (Group 2) was presented. The two groups were sacrificed following the completion of training and their brains removed. Again, RNA alone was presumably isolated from the individual brain homogenates via the phenol technique. The prepared extract was injected IP into light- and click-adapted rats. Those Ss receiving RNA from click-trained rats emitted more nonreinforced approach responses to the click than to the blinking light. On the other hand, more responses were emitted to the blinking light by Ss injected with an extract prepared from light-trained rats. The difference between the two groups was significant ($P < .001$). The authors concluded that responses of the recipients tended to be specific to the stimulus employed during training the donors. This would appear to rule out sensitization and motivational factors as explanations of Babich et al. (1965) results.

A further study also claimed success in obtaining the transfer of specific information using a discrimination task

and an RNA-specific phenol-extraction method. Fjerdingstad, Nissen, and Røigaard-Petersen (1965) trained a group of rats to traverse a double alley maze for delayed water reinforcement. Light-on and light-off were used as stimuli; only responses to the light-on side were reinforced. The experimental group received an intracisternal injection of a pooled RNA extract prepared from the trained rats' brains (RNA-light). The control group received an extract from unconditioned Ss (RNA-neutral). They reported a significant reduction in errors during acquisition to light-on in the experimental group.

In a second similarly designed study Røigaard-Petersen, Fjerdingstad, and Nissen (1965) obtained conditioned-extracts from two separate groups: RNA-light and RNA-dark. As well, four balanced testing conditions were employed; half of the experimental Ss receiving RNA-light were tested with light-on as the reinforced stimulus and half were tested with light-off as the reinforced stimulus. The other subgroup received RNA-dark; half were tested to light-on and half to light-off. The "transfer effect", a reduction in errors to criterion, was again observed. The reduction in errors during acquisition was obtained, however, only when the recipient's responses were reinforced to a stimulus opposite to that of the donor. When the same stimulus conditions prevailed no difference was obvious

from the control groups. No explanation was given for these results other than the suggestion that stimulus preference, pretest conditions, or certain inhibitory effects should be taken into account. Ungar (1966b), however, reports similar results, using a double alley maze and light-on and light-off as stimuli.

Reinis (1966) demonstrated the facilitated formation of a discriminated operant in rats as a function of injected brain homogenates prepared from similarly trained rats. The response conditioned was the opening of a door to gain access to a food cup within 25 sec. after the onset of a tone (Group 1) or a light (Group 2). The stimulus remained on for 20 sec. although only one response was reinforced. The donors and recipients were further subdivided into 16-hr. and 48-hr. food deprived groups. Upon the completion of training a brain homogenate was prepared from each donor's brain and injected into a recipient S. Forty-eight hours later testing began with each recipient tested to the same task as its donor. Control groups were provided by the injection of one group with a rat liver homogenate, another group received brain homogenate from naive Ss. The results of the donor Ss during training provided further control data.

The recipient Ss receiving trained-brain and starved for 48 hr. showed in comparison with the control groups: (1) A more rapid initial approach to the food cup, (2) A

greater number of reinforced responses, and (3) More "extra" responses interpreted as greater activity. The trained-brain recipients did not differ however, from the naive-brain recipients in the number of "extra" responses emitted. Thus, the noted increment in activity does not appear to be sufficient to explain the trained-brain recipients' greater number of reinforced operants. The latency of the responses did not differ between groups nor were there any significant differences as a function of the stimulus conditions employed. Since the transfer effect was not obtained in the 16-hr. deprived experimental group the relevance of the deprivation state of a recipient S is thus emphasized in the demonstration of the effect.

Rosenblatt, Farrow, and Herblin (1966) were successful in replicating Babich et al. (1965) results; they obtained a significant number of click-contingent-food-cup approaches when the brain-extract-injected Ss were compared with appropriate controls. In a second study, Rosenblatt, Farrow, and Rhine (1966) designed a comprehensive series of ten transfer experiments controlling for such factors as activity and general response enhancement. The tasks included classical and operant responses and a series of discrimination tasks. They reported that regardless of any generalized activating influence and regardless of whether positive or negative reinforcement was used, transfer was obtained in all

but two cases which showed marginal effects. The transfer seemed to be specific to the donor's learned task in all cases.

On the basis of the above data, it would appear that the transfer phenomenon is a real one. A wide variety of tasks seem susceptible to transfer while the recipient's post-injection behavior appears to be specific to information transferred interorganismically from a trained donor. However, the question of what is the "active transfer factor" remains unresolved. Babich et al. (1965), Jacobson et al. (1965), Fjerdingstad et al. (1965), and Røigaard-Petersen et al. (1965) favor an RNA interpretation. On the other hand, Ungar et al. (1965), Rosenblatt, Farrow, and Herblin (1966), and Rosenblatt, Farrow, and Rhine (1966) are explicit in favoring a polypeptide or small protein molecule. Reinis' extraction technique was not specific enough to delineate the nature of the transfer factor.

Three points weaken any hypothesis which suggests that RNA is the transfer factor. Ungar et al. (1965), Rosenblatt, Farrow, and Herblin (1966), and Rosenblatt, Farrow, and Rhine (1966) obtained the transfer effect when an extract was treated with ribonuclease (RNase). Ribonuclease renders RNA biologically inactive by breaking the molecule into its nucleotide components (Loring, Carpenter, & Roll, 1947). As well, Luttges, Johnson, Buck, Holland, and McGaugh (1966) made an attempt to

determine whether RNA contained in phenol prepared extracts could cross the blood-brain-barrier and be detected in the brain. Accordingly, several rats were injected with radioactively labelled RNA and sacrificed at various times up to 23 hr. after injection. The early concentrations of radioactivity were rapidly cleared from the peritoneum and no significant amounts of radioactivity could be found in the brain up to 23 hr. after the injection. Eist and Seal (1965) reported similar observations using rabbits and radioactively tagged yeast RNA. Thirdly, Ungar (1966) demonstrated that the active transfer factor is dialysable while it is known that RNA because of its large size is not.

It is proposed that the phenol extraction procedures employed by Babich, Jacobson, Fjerdingstad, and Røigaard-Petersen could not be expected to yield a transfer unless materials other than RNA were present in the extracts prepared. There is evidence to support this contention. However, an understanding of Babich's extraction technique in isolating RNA is crucial.

> "The tissue was placed in a cold mortar with 5 ml of phenol (90 per cent) and 5 ml of isotonic saline and was ground with purified sand for approximately 3 minutes at 0°C. The aqueous phase was carefully drawn off to avoid contamination with phenol or with the interphase. The aqueous phase was then brought up to a concentration of 0.1M $MgCl_2$, and 2 volumes of cold ethanol were added to precipitate the RNA. Precipitation time was 15 minutes. The suspension

> was centrifuged at 6000 rev/min for 15 minutes, after which the supernatant liquid was evaporated off, and the RNA was dissolved in 1.0 to 1.5 ml of isotonic saline. The amount of RNA was determined from the optical density at 260 mμ (εP = 7450 in 0.2M NaCl). The yields were in the range of 0.7 to 1.1 mg/ml per gram of tissue. Tests for protein with biuret (Layne, 1957) were negative and for DNA with diphenylamine were negative (Schneider, 1957). The RNA seemed relatively undergraded as measured by Sephadex chromatography."
>
> (Babich *et al.*, 1966, p. 656; italics mine).

It may be significant that Babich avoided "contamination" of the aqueous phase with phenol since both Ungar *et al.* (1965) and Rosenblatt, Farrow, and Herblin (1966) report that the transfer factor is soluble in phenol. If this is so, then Babich's positive results are only explainable by assuming that his extract was contaminated with phenol within which the active transfer factor resided. Several investigators mention that quantities of phenol do reside in extracts prepared according to Babich's instructions (Carlton, 1966; Rosenblatt, Farrow,& Herblin, 1966).

The majority of investigators which report negative findings while attempting to replicate Babich's results made particular efforts to isolate RNA and remove "contaminating" phenol. Such purification should jeopardize the chances of success if the active transfer factor resided in the phenol. Gross and Carey (1965) attempted without success on two occasions to replicate Babich's results. They point out in their second attempt that, "The biochemical procedures

were modified to eliminate phenol contamination and increase the yield of RNA". Gordon, Leanin, Leonhardt, and Gwynn (1966) were similarly unsuccessful; they employed the same phenol extraction technique used by Fjerdingstad et al. (1965) i.e., Laskov, Margoliash, Littauer, and Eisenberg (1959). Additionally, Luttges et al. (1966) sought to effect a "transfer of learning" using the phenol technique. However, they reported "...no evidence of 'transfer of learning'..." despite utilization of a wide range of tasks varying in complexity, usage of two different species of animals, and variations of the degree of the donor training, as well as the inclusion of testing intervals longer than those of Babich. Kimble and Kimble (1966) using the phenol technique were also unable to demonstrate any transfer effects. They designed a transfer test in which both positive and negative transfer predictions were possible. The donors consisted of rats trained on a T-maze black-white discrimination: 1/3 trained to black, 1/3 trained to white, and 1/3 given "mock trials" (untrained). The recipients were rewarded only for responses to the white arm of the maze. They concluded "...we view our results as indicating failure to find any transfer of training effects in our host rats as a result of intraperitoneal injection of RNA from rats trained in brightness

discrimination". Byrne et al. (1966) presented a summary statement of 18 experiments performed by various investigators in which no evidence of a transfer was found. The procedures employed: (1) The phenol extraction technique of Babich et al.(1965); (2) Several variations of the injection technique: pooled and unpooled donor's brains; and (3) Various training procedures: (a) Acquisition of an approach response like that of Babich et al. (1965); (b) A brightness discrimination in a T-maze; (c) A complex maze problem; (d) A conditioned emotional response (CER); and (e) Learning of a discrimination problem similar to that of Jacobson et al. (1965). Byrne et al. concluded, as has been suggested here, that the phenol extraction technique as a method of evaluating the possibility of memory transfer yields results which are not uniformly positive.

Biochemical analysis of prepared extracts which have been shown to yield the transfer effects has led several investigators to conclude that a small protein or polypeptide is actually the transfer factor. Ungar et al. (1965); Rosenblatt, Farrow, and Rhine (1966), and Rosenblatt, Farrow, and Herblin (1966) report that the transfer factor is soluble in polar water but insoluble in nonpolar acetone and 95 per cent ethanol. It is

dialysable as well. These characteristics are typical of peptides or small proteins. Ungar further supported this interpretation by incubating an active extract with crystalline cymotrypsin. The activity subsequently disappeared. Rosenblatt, Farrow, and Rhine (1966) verified Ungar's results. It is known that cymotrypsin hydrolyses proteins by splitting the protein into specific peptides (Fasold & Gundlach, 1963).

The above points implicate polypeptides as the probable "contaminating" material in Babich's extract. However, Babich reported that biuret tests for protein content in his extracts were negative. Objection may be made to Babich's usage of the biuret test on the grounds that this test is insensitive to concentrations of protein under 1 mg/ml. Rosenblatt, Farrow, and Herblin (1966) point out that a phenol prepared extract is contaminated with proteinaceous material (1.1 mg/ml.) as determined by the more sensitive Folin-Ciocalteau protein test (Lowry, Rosebrough, Farr, & Randall, 1951). The presence of protein may also be presumed for extracts obtained by Fjerdingstad et al. (1965) and Røigaard-Petersen et al. (1965) according to the phenol technique of Laskov et al. (1959). Laskov reports that the extract obtained by this method contains approximately 0.5 - 2.3% protein.

The present investigation was undertaken in order to develop an experimental procedure which would provide favorable conditions for the demonstration of a transfer effect, if such a phenomenon exists. The procedure incorporated the following:

(1) An extraction technique which was non-specific for any particular organic material.

(2) Control for possible motivational, sensitization, and general response enhancement interpretations.

(3) A sensitive behaviorial measure of learning.

The S^D-S^Δ operant discrimination schedule originally designed by Skinner (1938) consists of two alternately occurring intervals during which either the reinforcement-associated stimulus, S^D, or the nonreinforcement-associated stimulus, S^Δ, appear along a temporal continuum. D. M. Page (cited by Wilson & Kellar, 1953) suggested a modification of the schedule such that all responses whether to S^D or S^Δ reset the interval during which S^Δ is present. Such a schedule insures the extinction of responses to S^Δ; until responding to S^Δ is extinguished no responses are reinforced as S^D-onset never occurs. Wilson et al. (1953), Smith and Hoy (1954), and Carlton (1959) are in agreement as to the low inter- and intra-subject performance and learning variability of such a discriminated operant.

If the assumption is made that responding to the above schedule is under stimulus control then once learning of the discriminated operant is established -S^D elicits a bar press which is inhibited by S^Δ - these properties of stimulus control enable the testing of a differential "information transfer" via the brain-extraction-injection technique. For instance, "positive transfer" would be expected if the recipient were tested on the S^D-S^Δ schedule with S^D and S^Δ the same stimuli as those used in training the donor. Evidence for such a facilitating effect would be provided by several measures: reduced response latencies to S^D-onset and reduced response frequency in the presence of S^Δ. Each of these would summate to yield more rapid acquisition and a greater number of reinforcements over a fixed period. On the other hand, a "negative transfer" effect might be produced by simply reversing the donor-recipient S^D-S^Δ relationship. That is, if the recipient were tested with the same stimuli as the donor but in a contrasting relationship such that the donor's S^D became the recipient's S^Δ and vice versa, a negative or interfering effect would be expected. In detail the recipient would be expected to emit responses to the S^Δ while inhibiting responses in the presence of S^D with few responses being reinforced until extinction of responding in S^Δ began to occur.

Similarly if the donor were to receive no training there should be no transfer of relevant information to the recipient.

Reinis (1966) points out the relevance of controlling for altered activity as a function of an IP injection of brain homogenate. If motivational factors can be expected to be transferred or simply to interfere with the purity of a transfer effect it is best they be controlled. This may be accomplished in several ways. Besides equating all groups' levels of deprivation (donors' and recipients') a recipient control group may be included which receives a brain extract prepared from a donor which received no experience with the S^D-S^Δ schedule but was maintained on an equivalent deprivation regimen as trained donors. Further, a saline injected control group provides control over possible activation or depressant effects of the injection itself. Comparative information for all experimental groups may be weighed against the performance of the donors which act as noninjected controls.

In summary, it is hypothesized that:

I. A recipient will exhibit a facilitation of responding to S^D when it is the <u>same</u> as that of the donor; but an inhibition of responding when it is <u>opposite</u> to that of the donor.

II. A recipient will exhibit inhibition of responding during S^Δ when it is the <u>same</u> as that of the donor; but a facilitation when it is <u>opposite</u> to that of the donor.

Stimulus Control of The S^D-S^Δ Discriminated Operant

The predictions of the proposed experiment are dependent upon the assumption that the donor's responding is under stimulus control. Therefore, prior to the "memory transfer" attempt, an experiment was designed to evaluate the degree of stimulus control and also to determine the response pattern generated by such a schedule. Various difficulties inherent in evaluating stimulus control during conditioning by the method of successive stimulus presentations have been discussed by Skinner (1938), Smith and Hoy (1954), and Jenkins (1965).

Smith and Hoy have suggested that in using the discrimination procedure employed by Skinner (1938) in which the S^D and S^Δ are presented for a fixed interval, E (experimenter) cannot specify the exact nature of the stimulus that sets the occasion for the discriminated operant. They suggest, as does Skinner, two possible alternatives: ideally, S may be responding to the onset or presence of the reinforcement-associated stimulus (indicating stimulus control); or the S may be making a temporal discrimination thereby responding at intervals appropriate for maximizing reinforcement. If such temporal control is in operation then anticipatory responses (prior to S^D-onset) would most likely be the consequence of faulty temporal perception.

As an alternative to stimulus control, one could also argue that the organism was using the first nonreinforced response as a cue to initiate responding and the first nonreinforced response as a cue to terminate responding. Jenkins (1965) has discussed this possibility in terms of the correlation between antecedent reinforcement-nonreinforcement and stimulus presentation. He refers to this effect as a "cue effect" which confounds the measurement and attainment of stimulus control.

A further possibility which is actually a modified form of stimulus control is that the S may respond to the first stimulus change following the first nonreinforced response to S^{Δ}. In any of these cases: temporal control, the operation of a cuing effect, or responding to the first stimulus change following the first nonreinforced response to S^{Δ}, the consequent response patterning is the same for all intents and purposes as that generated by stimulus control. All predict a decreased response probability to S^{Δ} and an increased response probability to S^{D} thereby confounding the measurement of stimulus control. The prime concern here is to design a schedule which reduces, eliminates, or controls the effect of all of these influences with the exception of stimulus control.

The modification of the S^{D}-S^{Δ} schedule suggested by D. M. Page should eliminate any confounding cuing effect

as all responses reset the nonreinforced interval. By incorporating Page's modification into the S^D-S^Δ schedule the degree of stimulus control generated by the schedule may be evaluated in the following manner. First, a S is trained on the schedule using contrasting S^D and S^Δ stimuli and programming each response to reset a 20-sec. interval during which S^Δ is present and no bar presses are reinforced. When a S reaches asymptote on the schedule the S^D and S^Δ stimuli are interchanged midway through the following experimental session. If the onset of S^D initiated a response (stimulus control) during prereversal then following stimulus reversal S should emit more responses to reversed-S^D. On the other hand, if responding is under temporal control there should be no change in the response pattern following stimulus reversal. Further, if responding to the first stimulus change following a reinforced response was the controlling influence then following stimulus reversal there should also be no change in the response pattern with responses occurring only after reversed-S^D-onset.

Method

Subjects

Experiments I and II. Eight Sprague-Dawley albino rats were obtained from National Laboratory Animals Breeding Company(N.A.T. Lab. Co.; Edmonton, Alberta) for Experiment I. Eight hooded rats of the Royal Victoria strain purchased from The Quebec Breeding Farms, Inc. were used in Experiment II. The Ss in both studies were individually caged in a room separate from the experimental room under a 12-hr. light-dark cycle. The basic diet for the Ss consisted of Purina Laboratory Chow Checkers available ad lib in the home cages. Tap water was available in the home cages up to 22½ hr. prior to the initiation of training; thereafter, each S was maintained on a 23½-hr. water deprivation regimen and watered following each experimental session for ½ hr. The watering took place in upright watering cages separate from the home cages. During an experimental session, .02-ml. droplets of water reinforced the lever pressing response.

Experiment III. Seventy-five albino Wistar rats obtained from N.A.T. Lab. Co. were used in Experiment III which includes the replacement of three sick Ss. Subjects receiving the same experimental conditions were housed two to a cage with an ad lib supply of food blocks therein at all times. Water was never available in the Ss' home cages. As well, no intentional taming was provided the Ss. All

handling took place only while transporting the Ss to and from the experimental situation. A further modification involved the use of chloramphenicol (Animycetin®) powder dissolved in tap water provided at the watering cages. Approximately 64 mg/kg/day of chloramphenicol was obtained by each S i.e., the equivalent of 3.52 mg/kg/day of chloramphenicol activity. The solution was drunk readily and provided protection against disease during the experiment.

Apparatus

Experiment I. The conditioning apparatus consisted of three aluminum Skinner boxes measuring 14 in. long, 10 in. wide, and 9 in. high. The top of each box as well as the side panel to the right of the response lever was of plexiglas construction. The side panel, however, was covered with a piece of white bond paper in order to block a direct view of other aspects of the apparatus.

Depression of a response lever (Lehigh Valley Electronics Co., Model No. 1535) protruding 1 in. through the center of one wall, could deliver a reinforcement. The lever was located 2 3/4 in. above the floor of the box. A force of approximately 6 gm. was required to depress the lever which moved through a vertical distance of 2 mm. During shaping the E could activate the

dipper by means of a pushbutton switch. A water dipper (Lehigh Valley Electronics Co., Model No. 3151) delivered the reinforcement. The lip of the dipper projected up through the floor of a box and was located 5 in. to the right of the response lever.

Each box was placed inside a refrigerator cabinet in which the intensity of light, volume of circulating air, and extraneous sounds were controlled. Illumination of each Skinner box during Experiment I was provided by fluorescent bulbs on the ceiling of the experimental room 7 ft. above each refrigerator through a 1-sq. ft. opening which was sealed over with window glass. Luminance at the response lever within each box registered 25-ftc. using a Weston Illumination Meter (Leeds and Northrup Co., Model No. 756). An audio-generator (Health Corp., Model No. AD-1) delivered the appropriate stimulus, a 2000 cps. tone rated at 72 db. at the response lever by a Dawe Instruments Ltd. sound lever meter (Model No. 1400F). A L-pad speaker fader was used to correct daily fluctuations in the tone's intensity. The tone was delivered through a 3-in. Marsland speaker (Marsland Engineering Co., Model No. 3LS) mounted within the refrigerator, 12 in. to the right of the response lever. A switch enabled immediate reversal of stimulus conditions from tone-on to tone-off

or vice versa. A Fasco Independent Inc. silent fan (Model No. 50745-N) circulated 15 cu. ft. of air per minute and was mounted just below the speaker. Noise from the operation of programming and recording devices within the experimental room was masked by white-noise rated at 60 db. at the response lever with the refrigerator lid closed. However, there were no regular discriminative stimuli provided by external sources.

Eleven individual compartments of ¼-in plywood construction were used as upright watering cages. An ad lib quantity of tap water (Experiments I and II) or chloramphenicol powder mixed with tap water (Experiment III) was available in each cage. The cages were placed outside the experimental room and were covered at all times.

The recording devices consisted of two counters for each Skinner box, one to count the total responses for a S, and the other to count the number of reinforced responses. A Hunter KlockKounter (Model No. 120-A, Series D) was used to accumulate the reinforced-response latencies for each S during an experimental session. A series of contact and latching relays in conjunction with a Hunter Decade Interval Timer (Model No. 11-C) enabled each lever press to: (1) Reset a 20-sec. interval during which the water dipper was

inoperative, (2) Turn on the appropriate non-reinforced stimulus, (3) Add a tally to the total response counter, and (4) Pulse a Mnemotron Computer of Average Transients (Technical Measurements Corporation, Model No. 400-C). If an interval of at least 20 sec. transpired between responses, the equivalent of an inter-response time (IRT) of 20 sec., S^D immediately came on with a count added to the S^D-counter.

The computer was used to accumulate a frequency histogram of IRTs for all responses. The IRT interval of the histogram was 1.0 sec. for Experiments I and II and 5.0 sec. for Experiment III. The computer was set so that all responses with IRTs of 50 sec. or less were recorded. All responses with IRTs greater than 50 sec. were added to the first IRT interval (0.0 - 1.0 sec.). Therefore, the number of responses with IRTs exceeding 50 sec. was calculated as the difference between the computer's response tally for IRTs 20 - 50 sec. and the S^D counter's tally. The data for the first and second half of each session were stored in separate quadrants of the computer memory.

A Moseley X-Y recorder (Model No. 2D) printed an analog output from the computer in the form of an IRT frequency histogram. A Gralab Universal Timer (Model No. 171) was used for timing the 1-hr. experimental sessions.

Experiments II and III. Minor alterations in the apparatus were made for Experiments II and III. The window in each refrigerator cabinet was painted black and light-sealed. A small unpainted area approximately 1 in. by 2 in. permitted a restricted view within the refrigerator of the response lever and water dipper. This hole was uncovered only during shaping sessions.

The audio-generator was removed and white-noise generated directly into each cubicle via the 3-in. speaker. The L-pad was used to adjust the amplitude of the white masking noise to a level of 70 db.

A ½-in. diameter hole was cut in the aluminum wall to the left of the response lever of each Skinner box. A 6-v. bulb was placed just outside the hole permitting illumination (approx. 1 ftc.) of the response lever and the water delivery lip when all other light was eliminated from the apparatus. This light was used only during shaping and continuous reinforcement (CRF) conditioning.

A 40-w home appliance General Electric light bulb provided the discriminated stimulus. The bulb was placed below the speaker and fan and was located 12 in. from the response lever. Otherwise, the bulb was in a circuit similar to the tone-generator for Experiment I. Luminance at the response lever when the light was on registered

20 ftc. The white bond paper on the right plexiglas panel of the Skinner box diffused any direct light entering the apparatus.

Procedure

Experiments I and II. Both Experiments were designed to determine whether the responses emitted by a rat on an operant discrimination schedule (Carlton, 1959) are under temporal or stimulus control. Since more stringent control of visual stimulation could be accomplished than was possible with auditory stimulation (Experiment I), light-off and light-on were used as the stimulus conditions for Experiment II. As well, hooded rather than Sprague-Dawley albino rats (Experiment I) were employed in Experiment II since the latter strain could possess visual deficiencies because of the unpigmented eyes (Lashley, 1930). The design is described in terms of preliminary training and experimental testing.

Preliminary training included pretraining and discrimination training. A general outline of the preliminary training is given below:

1. Each S was placed on water deprivation 22½-hr. prior to the initiation of training.

2. Each rat was given daily 1-hr. shaping sessions in its assigned Skinner box for two days. If shaping was established in the first session, the second session consisted of 5 min. of CRF conditioning. The floor, dipper, and response lever were washed and dried after each session. Immediately following each experimental session throughout all phases of the Experiment the Ss were placed in a watering cage for ½ hr. The Ss were then returned to their home cages.

3. Three sessions of CRF conditioning were given. Each session was terminated after the S received 180 reinforcements.

4. On day six, 1-hr. daily discrimination training sessions commenced.

The daily discrimination performances were evaluated in terms of a discrimination index (DI; Carlton, 1959). The index expresses the frequency of reinforced responses as a proportion of the S's total reponses. Training continued until S performed with a DI of at least 70% for three consecutive sessions (80% for Experiment II).

The stimulus conditions employed during discrimination training were: one-half of the Ss received tone-on as S^D and tone-off as S^Δ (light-on and light-off, respectively, for Experiment II). The other half of the Ss received tone-off as S^D and tone-on as S^Δ (light-off and light-on, respectively, for Experiment II).

Each S was run during the same hour on consecutive days. At the beginning of each experimental session, the S was removed from its home cage, transported by hand to the experimental apparatus, and placed in its respective Skinner box. The refrigerator lid was then closed. During the days of training and testing, the first lever press of each session was reinforced and, thereafter, all responses were recorded with reinforcement delivered only when a response occurred after a 20-sec. response-free interval (IRT $\bar{>}$20 sec.) i.e., in the presence of S^D.

Experimental testing began the session following the day a S reached criterion. The testing session was divided into two half-hour periods. The first half differed in no way from the discrimination schedule which had previously been in effect for each S. However, in the second half of the session the stimulus conditions were reversed. That is, what had previously been S^D became the nonreinforced stimulus, reversed-S^D, while the previous S^Δ became the reinforced stimulus, reversed-S^D. Stimulus reversal took place without interruption of the schedule.

Pre- and post-reversal discrimination indices, latency indices, and IRT frequency histograms were used to evaluate the performance of all Ss during the two testing periods. The discrimination index expressed the frequency of reinforced

responses as a proportion of the Ss' total responses. The latency index stated the average response latency to S^D- or reversed -S^D-onset. Finally, the two IRT frequency histograms illustrated the frequency of responses which occurred within each of fifty one-second intervals after each response. For example, the frequency of responses within IRT 25 sec. indicates the number of times a response occurred between 25.0 and 25.9 sec. after any response during the period.

Experiment III. Experiment III was designed to test the hypothesis that specific control of a response may be "transferred" from one S (donor) to another S (recipient) by means of the injection of a brain extract.

The preliminary training may be discussed in terms of: (1) A 6-day watering regimen for all Ss, (2) The pretraining of the 36 donor and 36 recipient Ss, and (3) The discrimination training of 24 donor Ss.

Approximately 22½ hr. after the arrival of each S into the laboratory and placement in its home cage, S was removed from its home cage and placed into a watering cage for 1½ hr. where an ad lib supply of water was available. Following this watering period, S was returned to its home cage. This watering regimen was continued for a total of six days after which pretraining began.

All aspects of pretraining for donor and recipient Ss were identical to those carried out in Experiments I and II, except for 12 Ss of an untrained donor group (Donor Group #3). These Ss were maintained on the watering regimen for a total of 19 days and never received any discrimination training or shaping.

The 24 donor Ss receiving discrimination training were placed on the operant schedule similar to that employed in Experiment II. The training continued over eight daily 1-hr. sessions to insure a high level of performance. Records of each S's performance were obtained for each half of the first four sessions. Half of these 24 donor Ss received light-on as the S^D and light-off as S^Δ (Donor Groups #1 and #2); the other half of the Ss (Donor Groups #4 and #5) received the opposite stimulus combination. Groups #2 and #5 differed from Groups #1 and #4, respectively, in that they received two 2-ml. IP injections of 0.9% mammalian saline precisely 14 hr. prior to the initiation of discrimination training; eight days before their brains were extracted. The cold saline was injected into the right and left peritoneum in the same manner as the brain-extract-recipient Ss received their pretest injections. Thus, Groups #2 and #5 in addition to serving as donor groups also served as a control for the effects

of an IP injection of saline upon an S's subsequent performance on the S^D-S^Δ schedule. Regardless of which of the five treatment conditions a donor S received, each S was maintained on a schedule of 22½-hr. water deprivation for a total of 19 days. The various treatments are summarized in Table 1.

The experimental scheduling was arranged so that each S of the five donor groups would complete its assigned treatment condition the same day as its respective brain-extract recipient S completed its pretraining. Each recipient S was run in the same apparatus as its respective donor. Because of the large number of Ss and limited number of Skinner boxes the donor groups were run in the following sequence: Donor Groups #1 and #4, Days 1 - 27; Donor Group #3, Days 6 - 32; and Donor Groups #2 and #5, Days 21 - 47.

Approximately eight hours after a donor S completed its assigned treatment condition it was sacrificed by decapitation following anesthetization with ether. The whole brain was immediately removed from the skull. The brain was washed clean of any blood in 50 ml. of 0.9%-saline and all surface vessels removed. The brain was placed into a cold mortar containing 4 ml. of cold isotonic saline and 4 gm. of ignited sea sand. The

Table 1

The Five Treatment Conditions For Donor Ss of Experiment III

Donor Group	N	Pre-discrimination Treatment	Discrimination Training	
			S^{Δ}	S^{D}
#1	6	_____	Light-off	Light-on
#2	6	Saline injection	Light-off	Light-on
#3	12	Maintained on watering regimen 19 days		
#4	6	_____	Light-on	Light-on
#5	6	Saline injection	Light-on	Light-off

mixture was ground with a cold pestle for 3 min. Centrifugation of the homogenate was subsequently carried out in a Servall refrigerated centrifuge using 50-ml. cellulose nitrate tubes in rotor SS-34. The centrifugation scheme involved centrifuging the homogenate at 5,000 rpm. (3,020 g.) for the first 5 min. in order to reduce the probability of organic contents of a low molecular weight becoming trapped. The centrifuge was then turned up to 17,000 rpm. (34,800 g.) for 30 min. at 0 - 2 degrees C.

Upon completion of centrifuging the preparation was found to be separated into two distinct layers. The upper layer or supernatant could be removed easily and was poured off directly into a 2-cc. syringe; the quantity of the supernatant necessitated filling the syringe twice. Approximately 4 ml. of the supernatant was injected IP into a recipient S, 2 ml. to the right and 2 ml. to the left side. A 2-cc. sterilized syringe with a 3/4 in. 22-guage needle was used for the injections. All injections took place precisely 14 hr. prior to the recipient's first testing session.

Experimental testing of the recipient Ss began and proceeded in the same general fashion as the S^D-S^Δ training of the donor Ss. Of the 36 recipients, 12 received injections of an untrained rat's brain-extract

(Group A_2). Half of the remaining 24 recipients received rat brain-extract from individual Ss trained on the same discrimination schedule (Group A_1) and half from individual Ss trained on an opposite schedule (Group A_3). Thus, half of all recipient Ss were trained with light-on as S^D (B_1) and half with light-off as S^D (B_2). The 12 donor Ss which received saline injections were equally distributed across all recipient groups. See Table 2 for details of the specific donor-recipient relationships and predictions. Recall that the two donor groups (uninjected and saline injected) also act as control groups. The performances of each S were recorded for each half of the first four sessions following injection. Thus, eight half-hour IRT frequency histograms were obtained for each S. Latency indices were also obtained for each S.

Table 2

Summary Statement of Donor-Recipient Relationships and Predictions Where A Refers to the Prediction Group and B Refers to the S^D Stimulus Condition for Experiment III

Recipient's Treatment	N	Donor's S^D	Recipient's S^D	Prediction
A_1-B_1	6	Light-on	Light-on	Positive
A_1-B_2	6	Light-off	Light-off	Positive
A_2-B_1	6	No training[a]	Light-on	Neutral
A_2-B_2	6	No training[a]	Light-off	Neutral
A_3-B_1	6	Light-off	Light-on	Negative
A_3-B_2	6	Light-on	Light-off	Negative

[a] Maintained for 19 days on watering regimen - Donor Group #3

Results

Experiment I

Examination of the sessions to criterion and the pre- and post-reversal IRT frequencies indicated no discernable differences in performance as a function of either S^D-tone-on or S^D-tone-off. Therefore, the stimulus conditions were considered equally effective discriminative stimuli and the results of all Ss were combined for analysis.

The criterion for acquisition (DI = 70% for three consecutive sessions) was attained in a mean of 10.7 sessions (S.D. = 2.9 sessions). One S did not reach criterion within twenty sessions and was discarded from the Experiment. There were numerous instances of performance decrements by individual Ss from one session to another as measured by the DI. As well, the variation between Ss' performances was considerable.

The prereversal data during testing is of initial concern. The dependent variable was the total number of responses for all Ss (N = 7) with IRTs of 0 - 1, 1 - 2, ..., and 39 - 40 sec. The number of responses with IRTs greater than 40 sec. was also determined. As indicated in Figure 1 the Ss tended to respond primarily in the presence of S^D during prereversal. The greatest proportion (389/634 res. = 61.4%) of the total responses had IRTs of 20 - 25 sec. i.e., within 5 sec. after S^D-onset. The DI

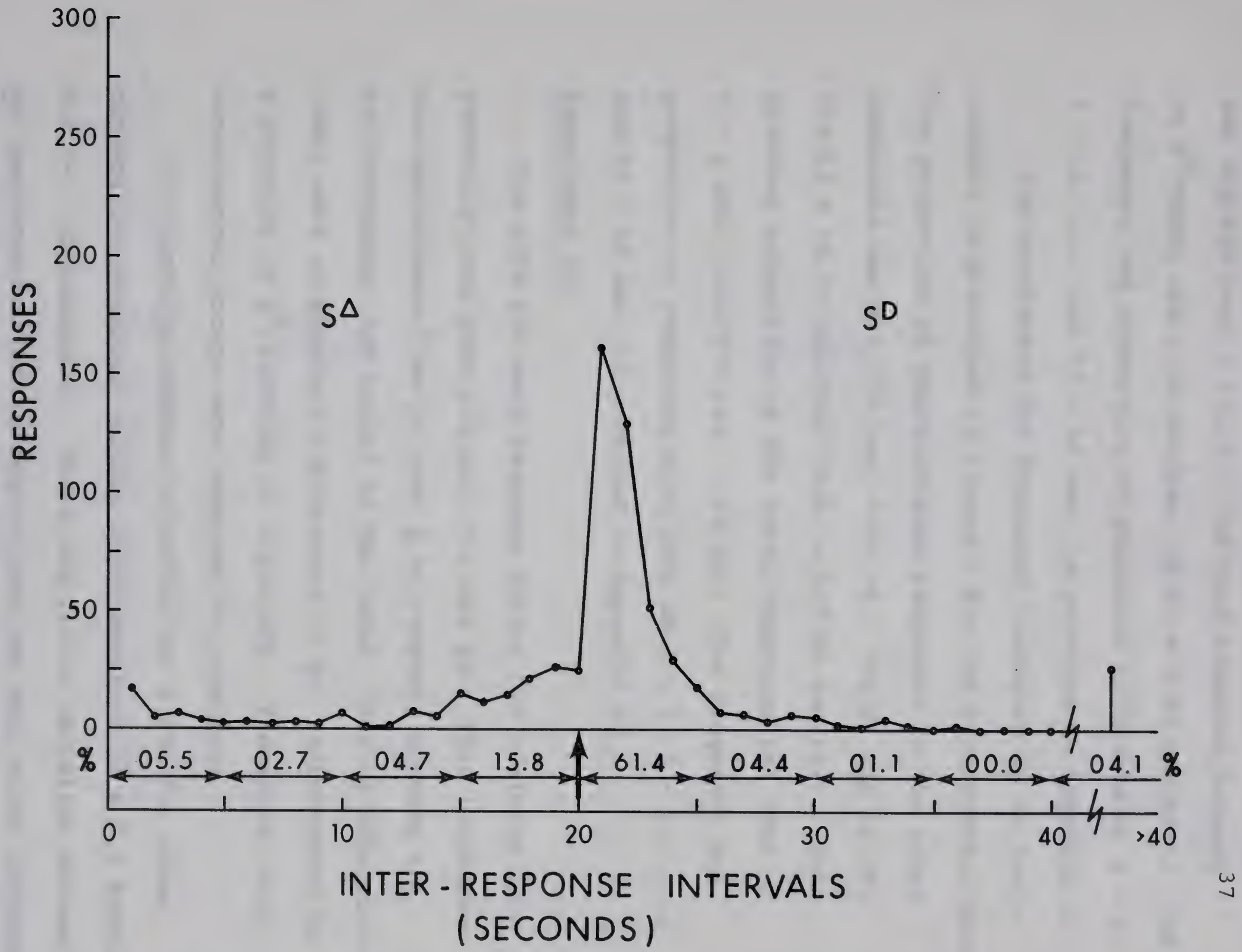
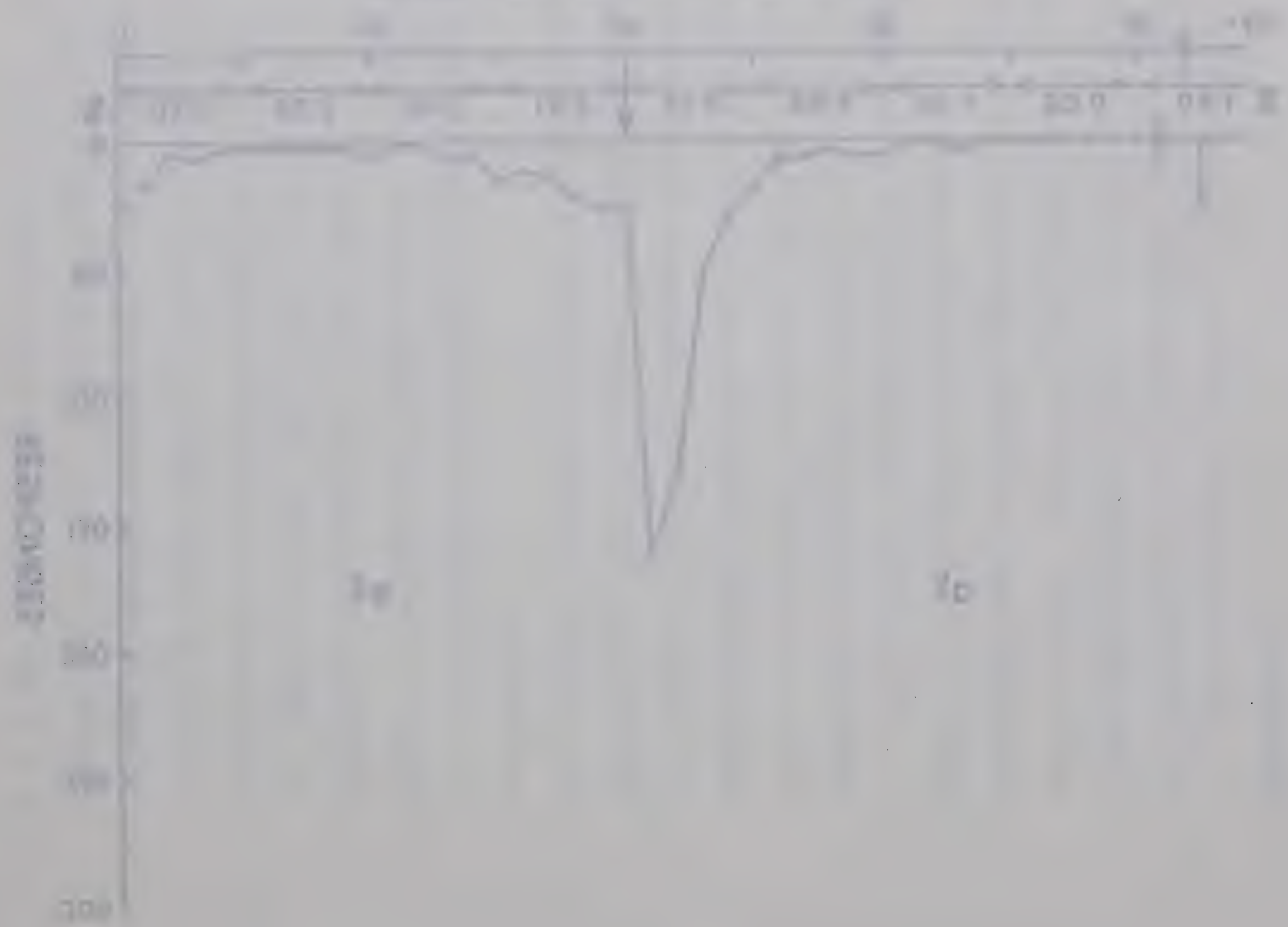


FIGURE I RESPONSE FREQUENCIES AND PROPORTIONS FOR EACH IRT DURING PREREVERSAL -- EXPERIMENT I (N=7)



was 452/634 res. = 71.2%. The mean response latency to S^D-onset was 1.98 sec/res. (S.D. = 0.80 sec/res). The frequency and proportion of responses with IRTs of 0 - 5, 5 - 10, ..., and 35 - 40 sec. is provided in Appendix A.

The cumulative IRT frequency histogram up to forty seconds is presented in Figure 2 for the postreversal data. The proportion of nonreinforced responses to the total responses was 632/759 res. = 83.3%. The DI was 16.7%. The LI = 50.06 sec/res. (S.D. = 115.00 sec/res). The greatest proportion of the total responses had IRTs of 0 - 5 sec. (420/759 res. - 55.3%). The frequency and proportion of responses with IRTs of 0 - 5, 5 - 10, ..., and 35 - 40 sec. is provided in Appendix B.

Experiment II

The IRTs for each response during CRF training were generally less than 5.0 sec. for all Ss. This indicates the approximate time it took S to respond, consume the reinforcement, and return to the lever. During acquisition, there were no apparent differences in Ss' performances as a function of S^D-light-on or light-off. Therefore, both experimental groups were combined for analysis.

All eight Ss reached criterion (DI = 80% for three consecutive sessions) within eight sessions ($\bar{X}$ = 5.5 sessions; S.D. = 1.2 sessions). There was little variation between Ss' performances during acquisition and only minor instances

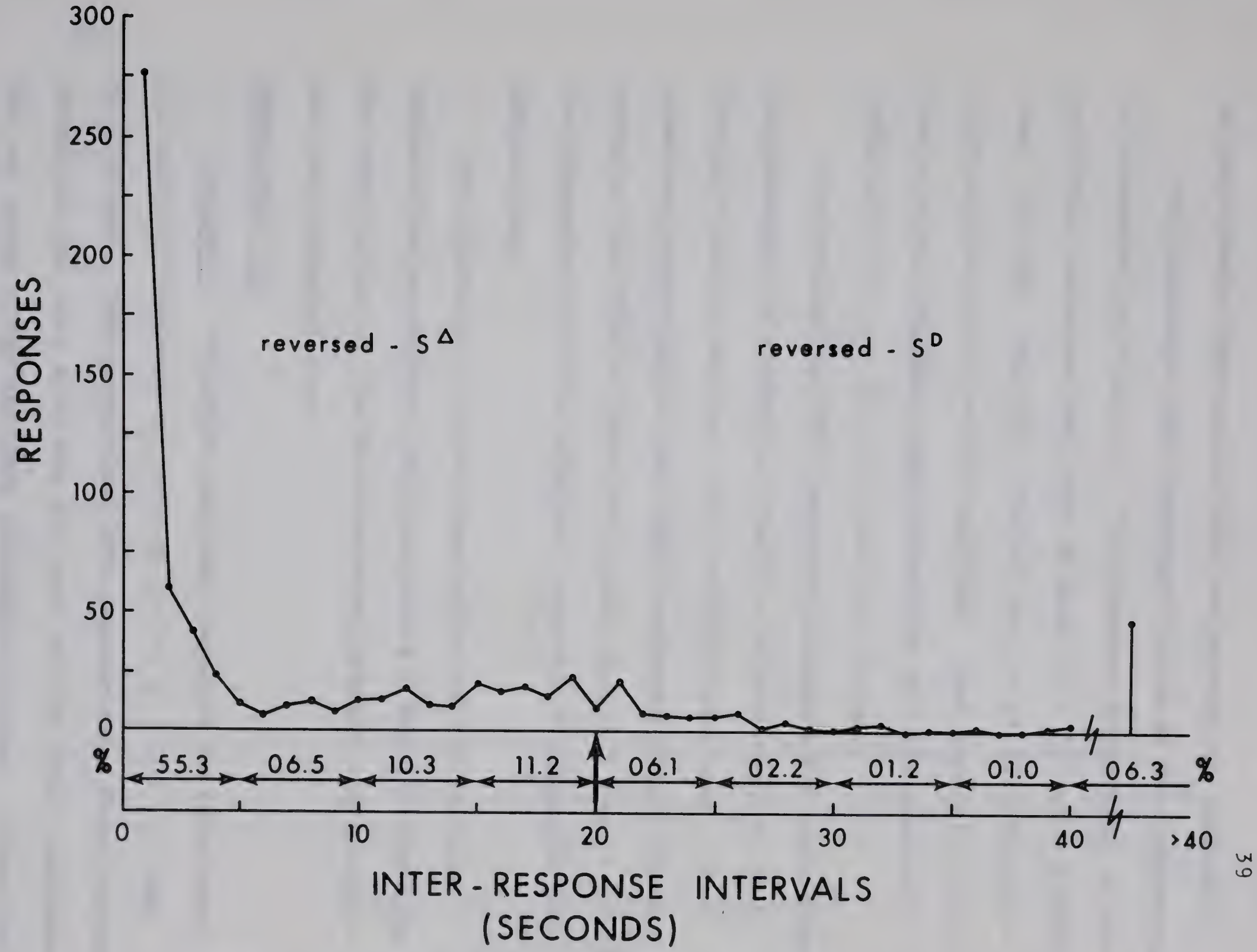
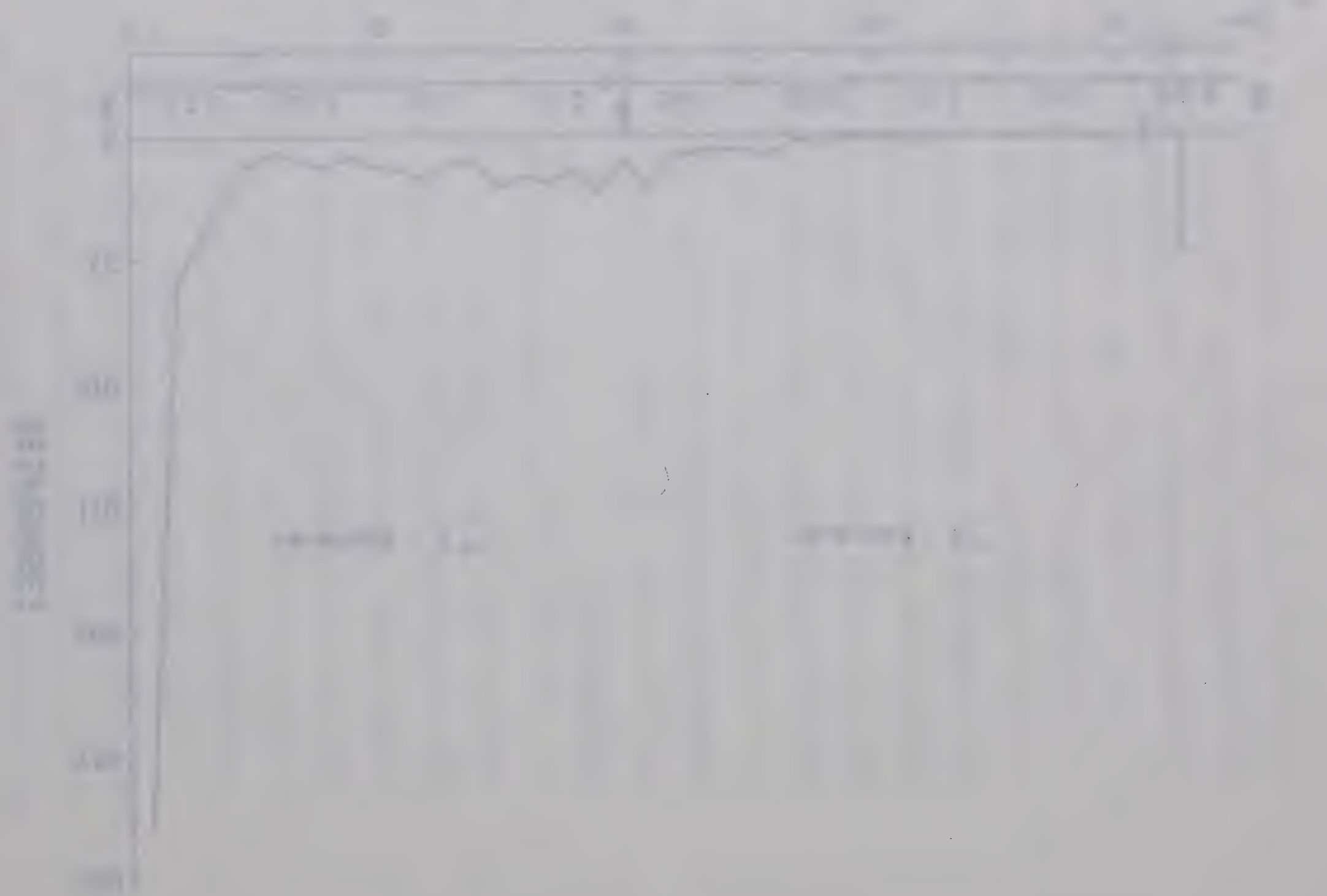


FIGURE 2 RESPONSE FREQUENCIES AND PROPORTIONS FOR EACH IRT DURING POSTREVERSAL -- EXPERIMENT I (N=7)



of performance decrements from one session to another.

The cumulative IRT frequency histogram for prereversal is presented in Figure 3. The greatest proportion of the Ss' total responses had IRTs of from 20 - 25 sec. (514/655 res. = 78.5%). The DI = 576/655 res. = 87.9%. The LI = 2.38 sec/res. (S.D. = 0.73 sec/res). See Appendix C for the original frequency and proportion of responses with IRTs of 0 - 5, 5 - 10, ..., and 35 - 40 sec.

The cumulative IRT frequency histogram for postreversal is presented in Figure 4. The DI = 83/632 res. = 13.4%. The LI = 102.63 sec/res. (S.D. = 874.74 sec/res). The greatest proportion of the total responses during post-reversal had IRTs of from 0 - 5 sec. (344/632 res. = 54.4%). See Appendix D for the original response frequencies and proportions.

See Appendix E for a presentation of the various comparative parameters for Experiments I and II regarding the acquisition of the discriminated operant and the pre- and post-reversal data.

Experiment III

Analyses. The prime dependent measure in Experiment III was the frequency of IRTs of 0 - 5, 5 - 10, ..., and 35 - 40 sec. Responses with IRTs greater than forty seconds accounted for less than one per cent of the total responses for any group of Ss and were therefore ignored. Since the

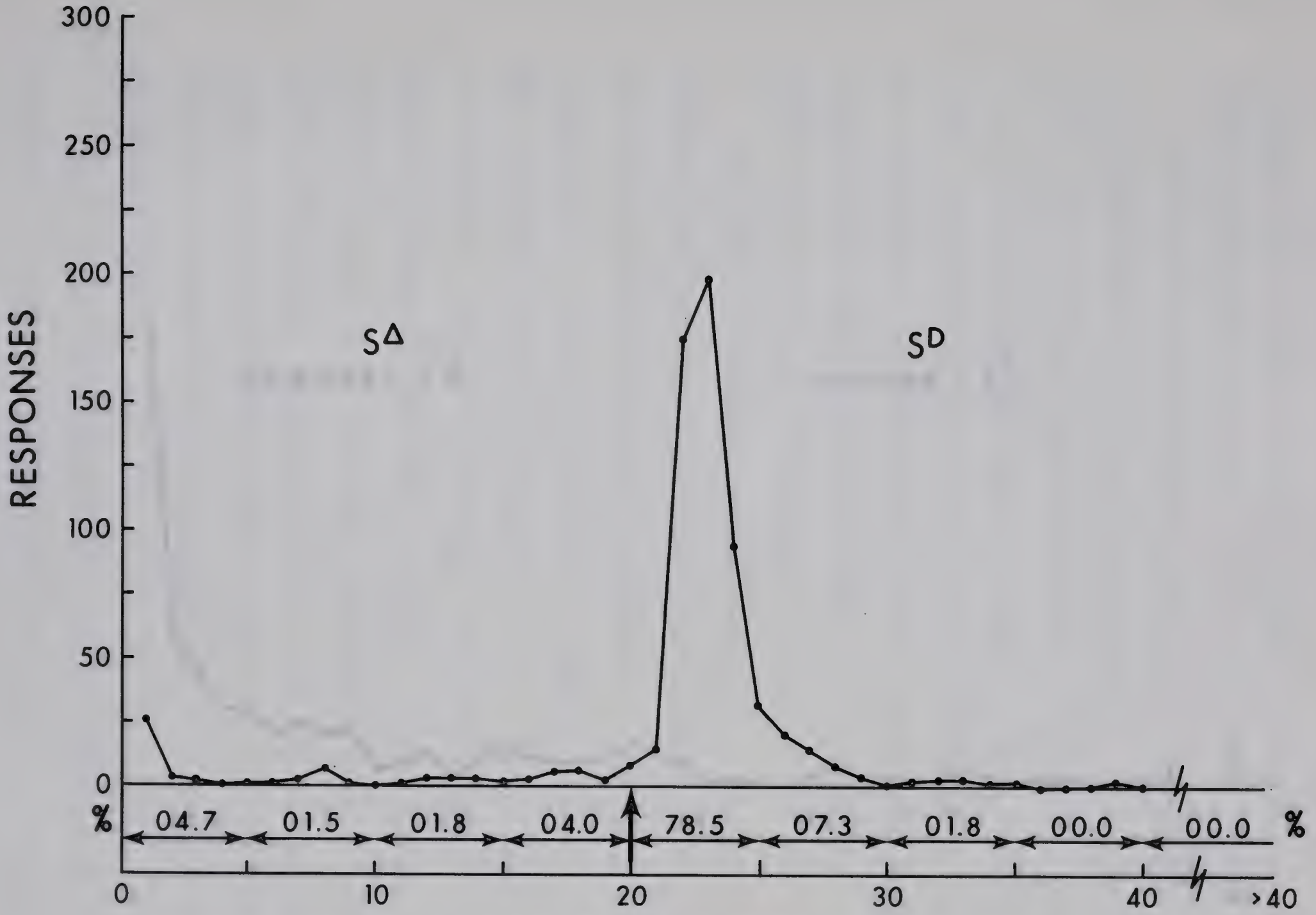
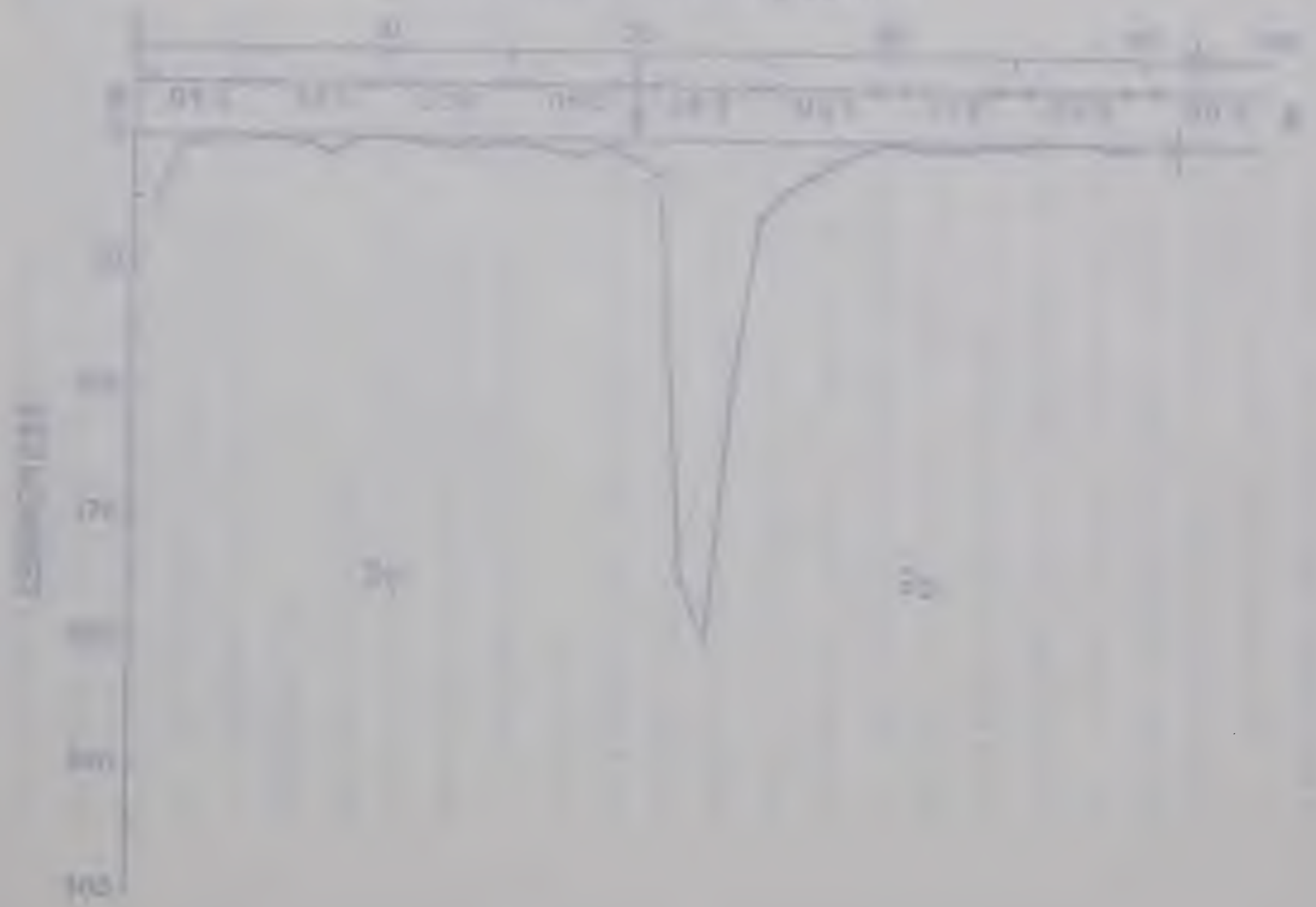


FIGURE 3 RESPONSE FREQUENCIES AND PROPORTIONS FOR EACH IRT DURING PREREVERSAL -- EXPERIMENT II (N=8)



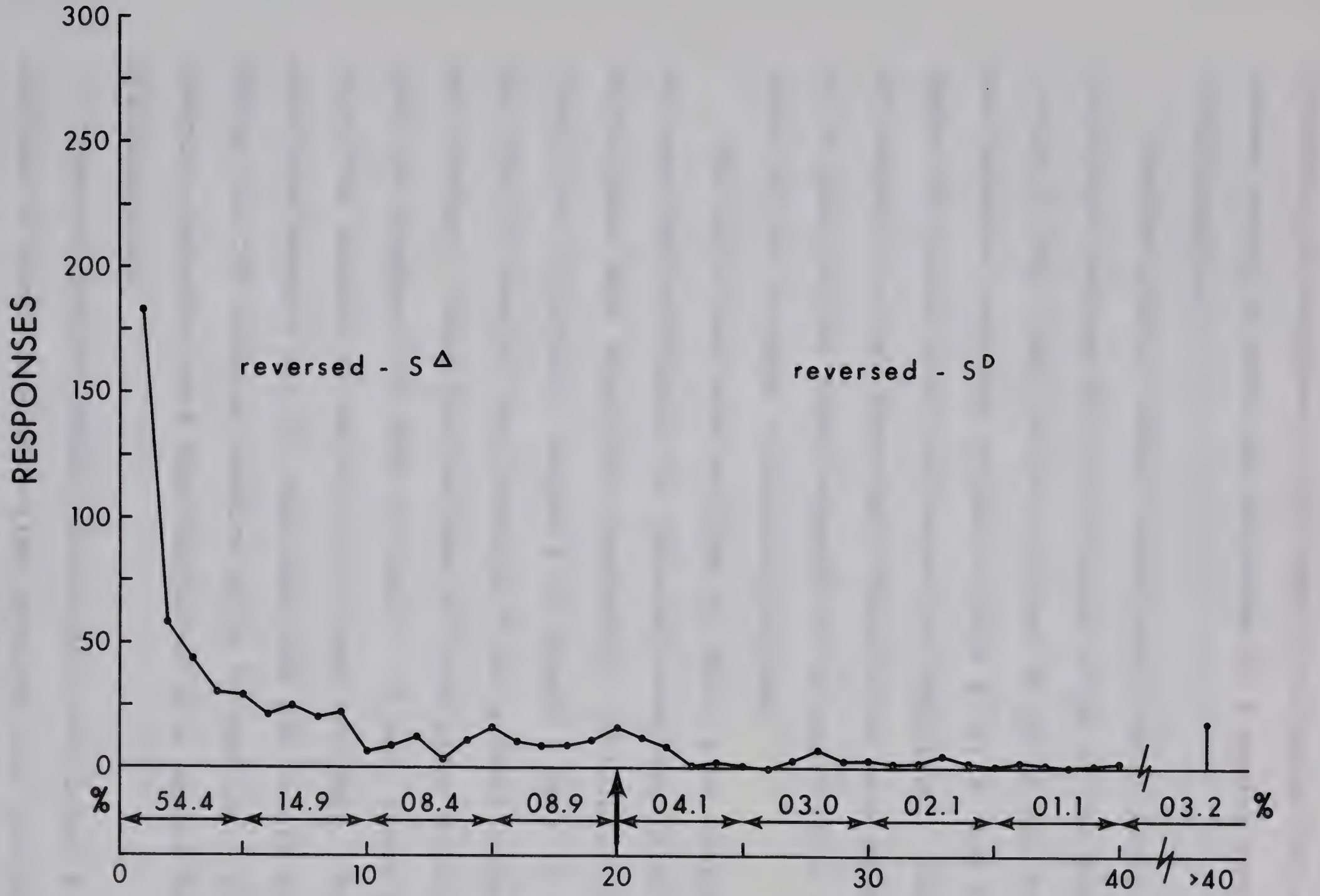


FIGURE 4 RESPONSE FREQUENCIES AND PROPORTIONS FOR EACH IRT DURING POSTREVERSAL -- EXPERIMENT II (N=8)

frequency of responses within each five-second IRT was skewed each S's score was subjected to a square root transformation.

Pearson product moment correlation coefficients were calculated between the performances of Ss of the donor groups on Day 4 and the performances of Ss of the Positive and Negative recipient groups on Days 1, 2, 3, and 4. Each donor was paired with its respective recipient. Two sets of correlations are presented, those which were expected to be positive and those expected to be negative on the basis of the transfer hypothesis proposed.

The data also were analyzed by split-plot analyses of variance and covariance for repeated measures. Five sources of variance were separated: Treatments (5 levels), Stimulus Conditions (2 levels), Subjects (6 levels), Days (4 levels), and IRTs (8 levels). See Appendix F for a description of each factor. Since the analyses of covariance were performed upon the frequency of IRTs of from 0 - 5 sec. there were only four sources of variance for these analyses. The covariate measure was S's response rate per minute obtained during the CRF training session prior to testing. The covariate measures were also subjected to a square root transformation.

Pearson product moment correlation coefficients and analyses of covariance were also obtained upon the daily

average response latencies to S^D-onset (LIs). The covariate measures were again each S's pretesting response rate per minute. The response latencies were transformed to speed measures by taking their reciprocal.

Correlation data. Experiments I and II indicated that the most sensitive indicants that responding was under stimulus control were: (1) The frequency of 0 - 5 sec. IRTs and (2) The frequency of IRTs of 20 - 25 sec. Therefore, donor-recipient performance correlations are presented for only these two five-second IRTs.

Positive correlations were expected when the same stimulus conditions prevailed for the recipients (Days 1 - 4) and their donors (Day 4). Therefore, each Negative recipient S's frequency of IRTs of 0 - 5 and 20 - 25 sec. was paired with its respective donor S's frequency of IRTs of 20 - 25 and 0 - 5 sec., respectively. These correlations are shown in Table 3. For the Negative recipient group, low positive (nonsignificant) correlations were obtained on five of the eight correlations presented for the four days of testing. The Positive recipient group's frequency of responses with IRTs corresponding to their donors were found to be consistently positively correlated. Significant positive correlations were obtained for IRTs of 20 - 25 sec. on Days 1 - 3. See Appendix G for the correlations obtained on all eight IRTs for each Day for each recipient group with the

Table 3 Obtained Correlation Coefficients Between Donor's and Their Respective Recipient's IRT Frequencies Where Positive Correlations Were Expected

Day	Treatment	Recipient's: IRT 0 - 5 sec.	IRT 20 - 25 sec.
1	Positive	+.096	+.688**
	Negative	+.137	+.006
2	Positive	+.485	+.822***
	Negative	+.072	+.011
3	Positive	+.326	+.591*
	Negative	-.208	-.072
4	Positive	+.260	+.344
	Negative	+.390	-.388

*P<.050
**P<.010
***P<.005

IRTs paired for the same stimulus conditions.

Negative correlations were expected between the donors and their recipients when the opposite stimulus conditions prevailed for the two groups. Each Positive recipient S's IRTs of 0 - 5 sec. and 20 - 25 sec. was paired for each day with its respective donor S's IRTs of 20 - 25 sec. and 0 - 5 sec., respectively, for Day 4. As shown in Table 4, consistently negative (though nonsignificant) correlations were obtained in each instance over the four days of testing. The Negative recipient group's frequency of responses with IRTs corresponding to their donors were generally negatively correlated; six of the eight correlations were negative but none were significant. The obtained correlations which were expected to be negative are presented in Appendix H for all eight IRTs and all four days for each recipient group.

Scatter diagrams of the significant correlations are presented in Appendix I. The correlations were suspected to be spurious as a function of differential responding to the two stimulus conditions or within the three testing apparatuses. Recalculated correlations for the Positive recipient group with its donors (same stimulus conditions) are presented in Appendix J. The correlations were reduced when the stimulus conditions were held constant but were increased when the apparatuses were held constant. Despite

Table 4 Obtained Correlation Coefficients Between Donor's and Their Respective Recipient's IRT Frequencies Where Negative Correlations Were Expected

Day	Treatment	Recipient's IRT 0 - 5 sec.	IRT 20 - 25 sec.
1	Positive	-.083	-.178
	Negative	-.388	-.068
2	Positive	-.562	-.331
	Negative	-.004	-.189
3	Positive	-.282	-.334
	Negative	+.148	-.053
4	Positive	-.370	-.420
	Negative	-.076	+.158

the reductions, IRTs of 20 - 25 sec. remained significant for Days 1 ($P<.05$) and 2($P<.025$)but not for Day 3.

The correlation coefficients obtained between the donor's (Day 4) and their respective recipient's (Days 1 - 4) LIs are presented in Table 5 for the Positive and Negative recipient groups. Although none of the correlations were significant the Positive group showed positive correlations on all four days of testing while the Negative group showed low negative correlations on two of the four days (Days 1 and 2) and low positive correlations on the other two.

Analyses of Variance and Covariance. The data for control donor groups (uninjected and saline injected) and experimental recipient groups (Positive, Neutral, and Negative treatments) were subjected to separate analyses due to the nonindependence of the donors and recipients as indicated by the correlation data.

A summary of the analyses of variance upon the control and experimental data is presented in Tables 6 and 7, respectively. The control data yielded no significant interactions which were not also significant for the experimental data. The form of the interactions was also the same. The control group's Treatments main effect, however, was significant ($P<.05$) and indicated that the saline injected group showed a higher total response frequency

Table 5 The Correlation Coefficients Between the LI's of Donors (Day 4) and Their Recipients (Days 1 - 4)

Day	Treatment	Correlation
1	Positive	+.316
	Negative	-.054
2	Positive	+.473
	Negative	-.135
3	Positive	+.108
	Negative	+.153
4	Positive	+.204
	Negative	+.004

Table 6

Summary of the Analysis of Variance for the Control Groups' Response Frequencies per IRT in Experiment III

Source of Variance	Sum of Squares	d.f.	Mean Square	F
Treatments (A)	18.65	1	18.65	4.54*
Stimulus Conditions (B)	3.33	1	3.33	
A x B	0.16	1	0.16	
Error: Pooled Subjects(C)	82.22	20	4.11	
Days (D)	379.64	3	126.55	143.92**
D x A	3.77	3	1.25	
D x B	2.11	3	0.70	
D x A x B	3.01	3	1.00	
Error: D x C	52.76	60	0.88	
IRT (F)	3611.79	7	515.97	168.38**
F x A	23.28	7	3.33	
F x B	67.29	7	9.61	3.14**
F x A x B	11.75	7	1.68	
Error: F x C	429.02	140	3.06	
F x D	2204.68	21	104.99	149.78**
F x D x A	11.91	21	0.57	
F x D x B	18.60	21	0.89	
F x D x A x B	8.59	21	0.41	
Error: F x D x C	294.40	420	0.70	
Total	7226.96	767		

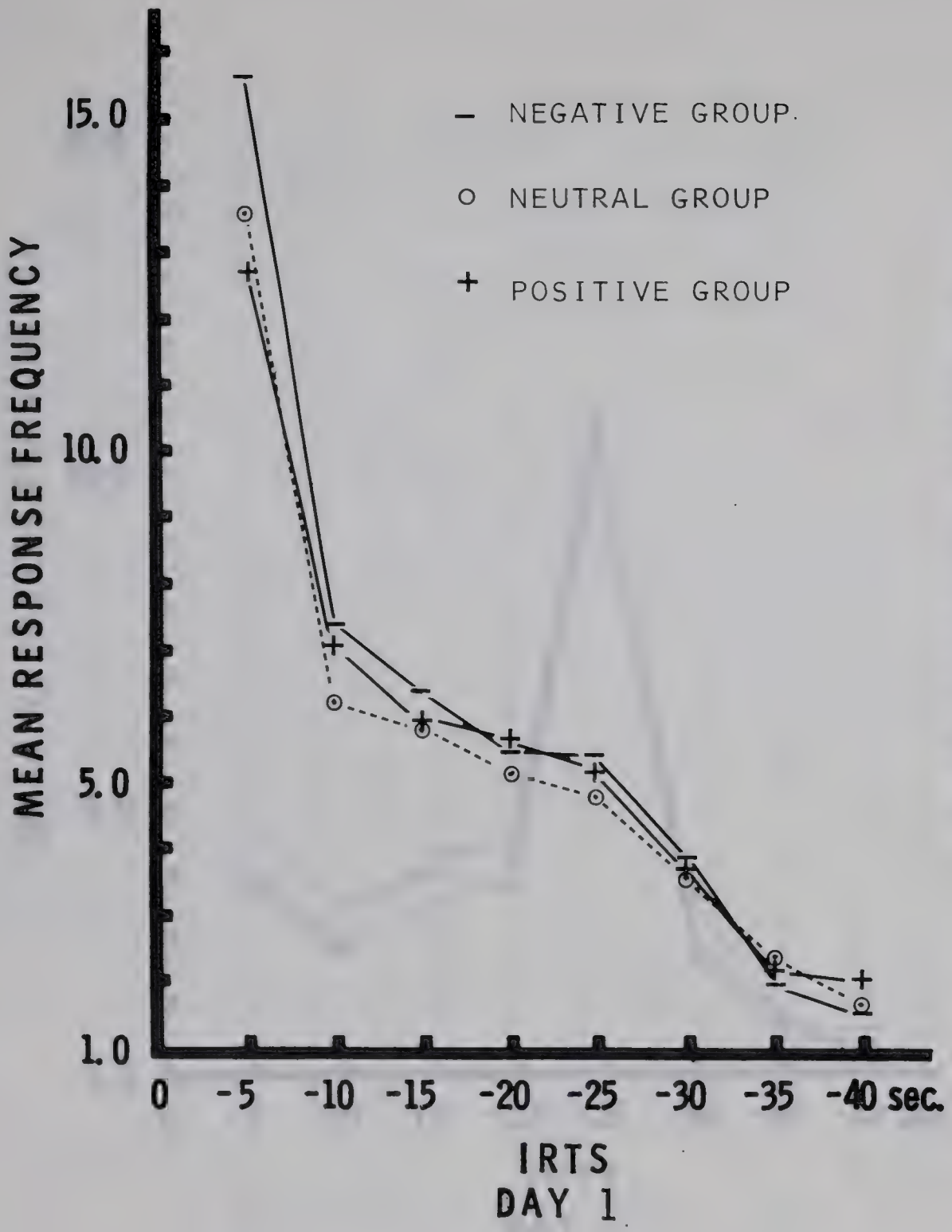
*$P < .050$
**$P < .005$

Table 7

Analysis of Variance for the Three Experimental Groups' Response Frequencies per IRT in Experiment III

Source of Variance	Sum of Squares	d.f.	Mean Square	F
Treatments (A)	14.01	2	7.00	
Stimulus Conditions (B)	32.54	1	32.54	11.99*
A x B	17.63	2	8.82	
Error: Pooled Subjects(C)	81.41	30	2.71	
Days (D)	598.82	3	199.61	177.27*
D x A	7.20	6	1.20	
D x B	45.98	3	15.33	13.61*
D x A x B	2.73	6	0.45	
Error: D x C	101.34	90	1.13	
IRT (F)	6360.08	7	908.58	426.57*
F x A	46.05	14	3.29	
F x B	166.82	7	23.83	11.19*
F x A x B	48.41	14	3.46	
Error: F x C	477.30	210	2.13	
F x D	3314.83	21	157.85	250.71*
F x D x A	50.84	42	1.21	1.92*
F x D x B	71.84	21	3.42	5.43*
F x D x A x B	28.27	42	0.67	
Error: F x D x C	396.66	630	0.63	
Total	11832.76	1151		

*P<.005



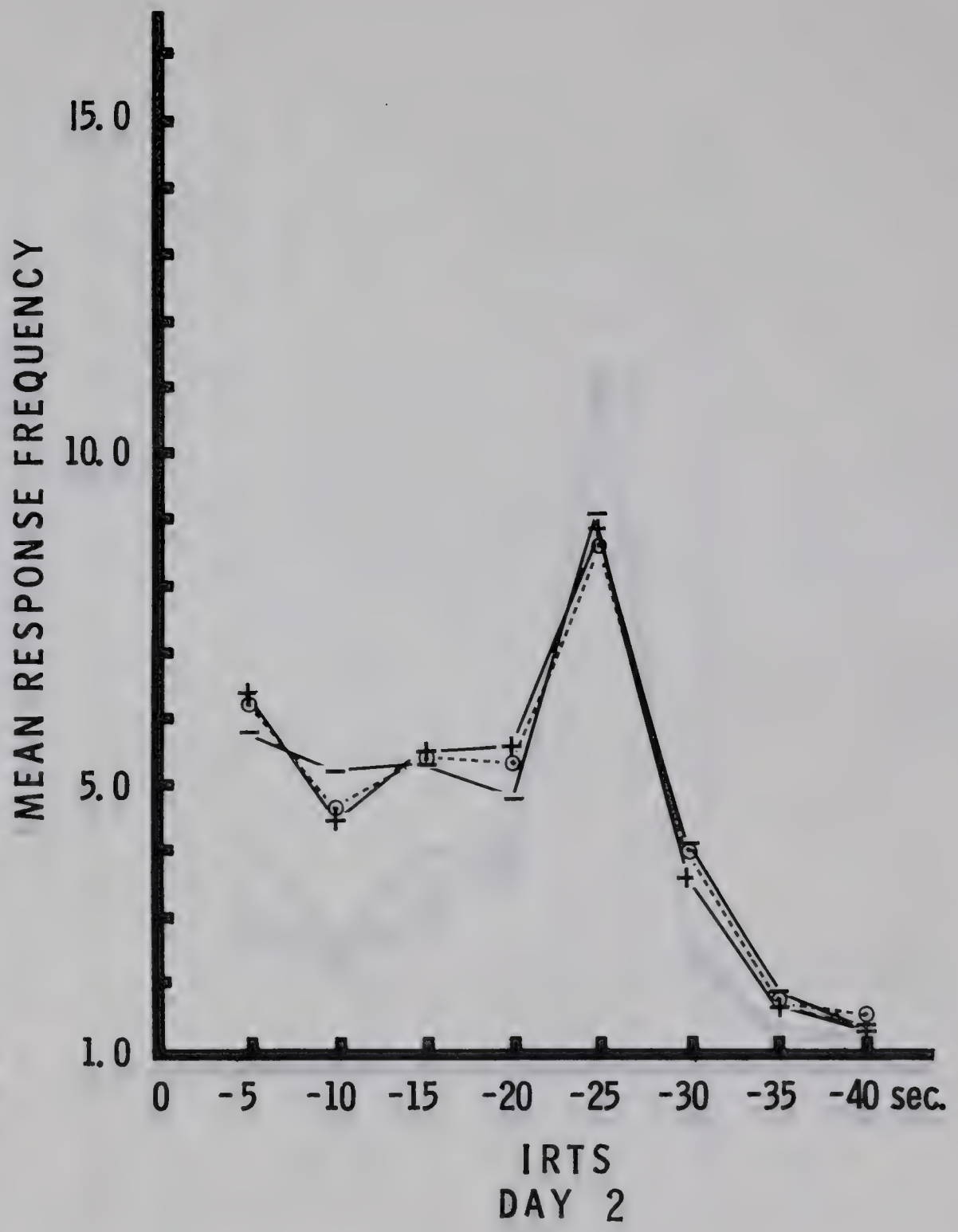
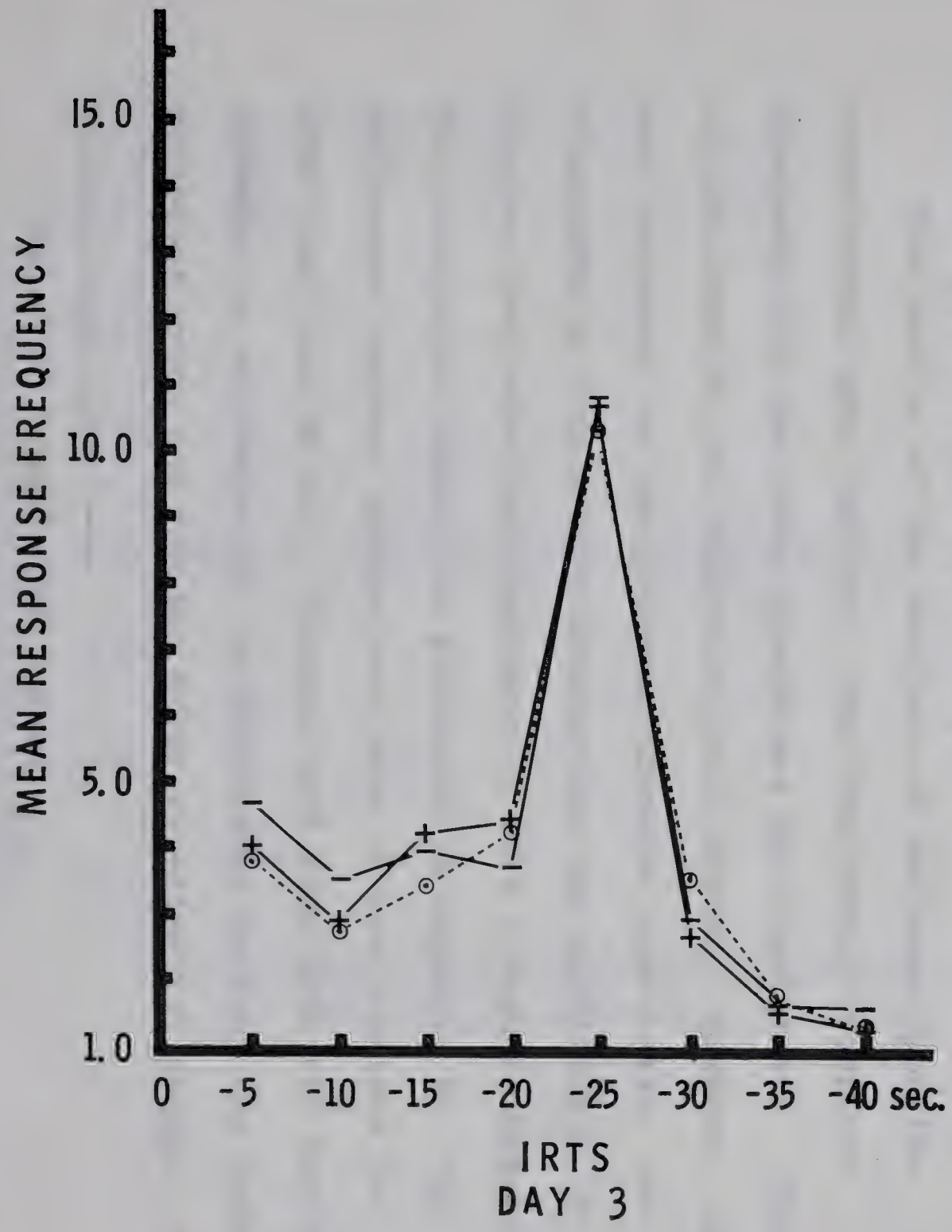


FIGURE 5A AND 5B TREATMENTS X IRTS INTERACTION FOR EACH DAY OF TESTING-- EXPERIMENTAL RECIPIENT GROUPS -- EXPERIMENT III



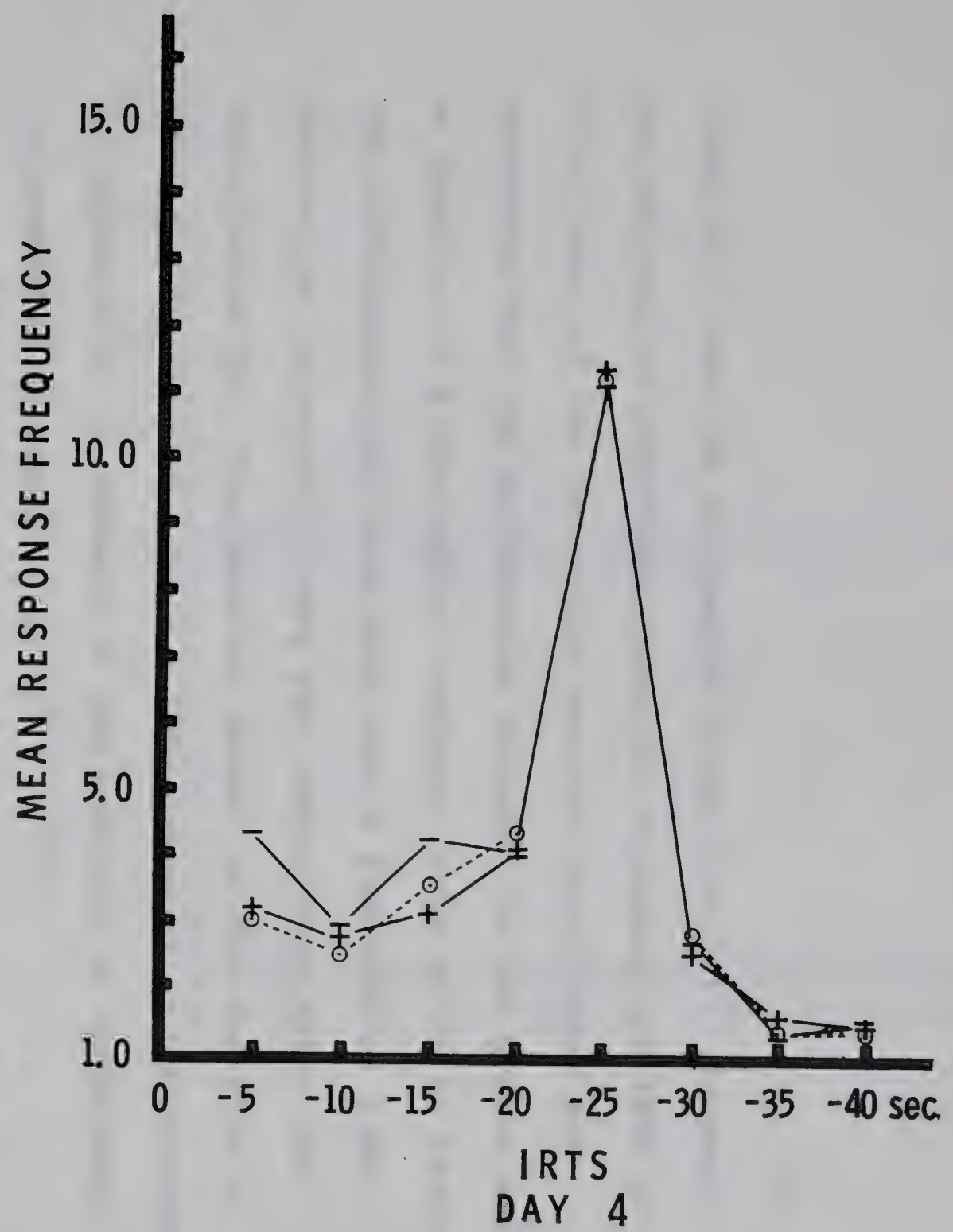
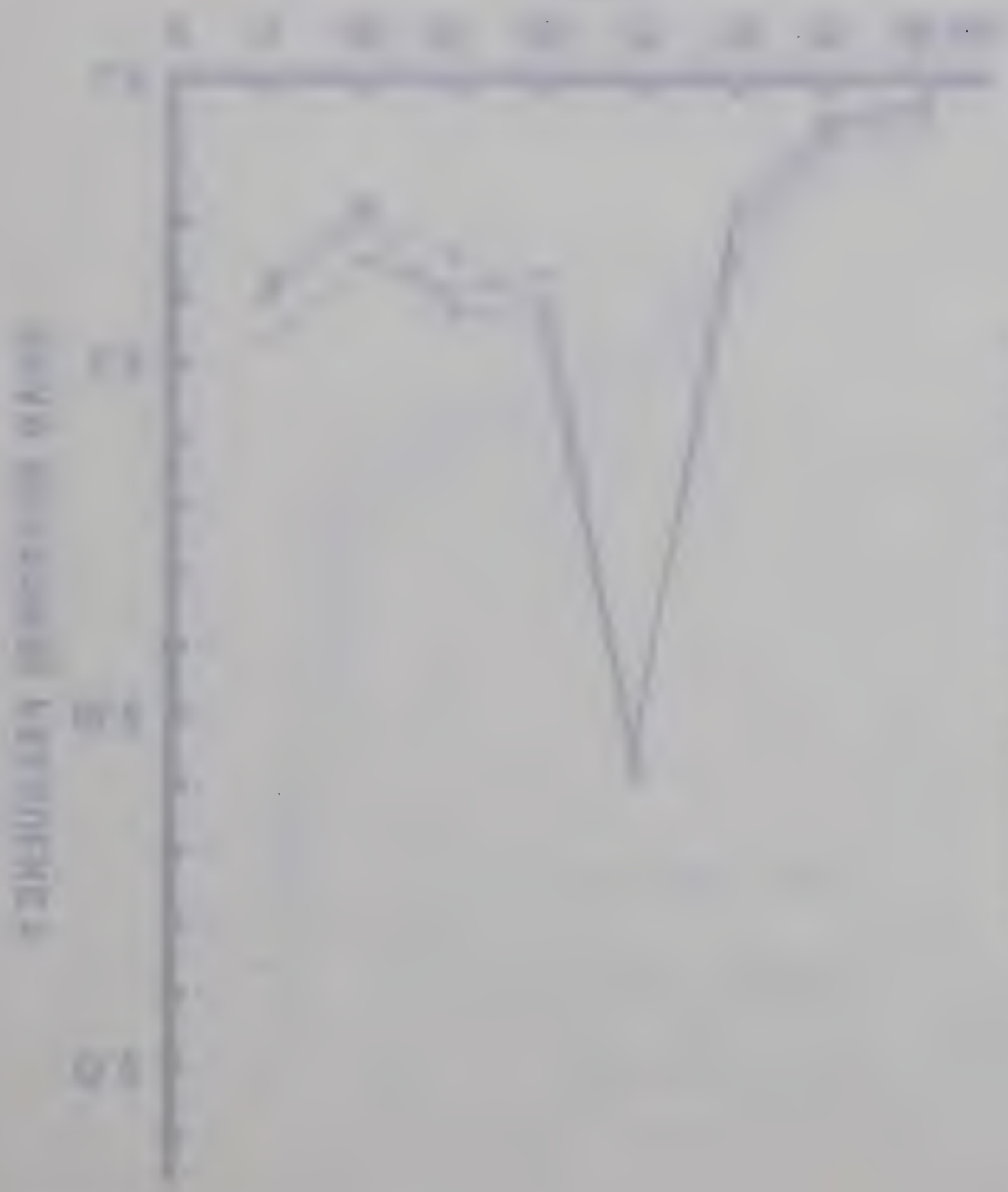


FIGURE 5C AND 5D TREATMENTS X IRTS INTERACTION FOR EACH DAY OF TESTING -- EXPERIMENTAL RECIPIENT GROUPS -- EXPERIMENT III



(148.54)[1] than the uninjected group (138.59). However, an analysis of covariance upon the frequency of IRTs of 0 - 5 sec. of the two groups was not significant and suggests that the differences between the two groups was a function of differential response rates prior to testing. The differences may have also been a function of time; the saline injected Ss were run twenty days after the noninjected Ss. The sums and means for the analysis of variance Treatments x Days x IRTs interaction are presented in Appendix K. A summary of the analysis of covariance is presented in Appendix L.

The results of the analysis of variance upon the experimental data indicate that the overall response frequency for the three groups was not significant. This is indicated by the Treatments main effect. However, and most relevant for the proposed transfer hypothesis, the Treatments x IRTs x Days interaction was significant ($P<.005$). Figures 5a-d show the Treatments x IRTs x Days interaction. The interaction means are available in Appendix M. The difference between groups over Days is most obvious for IRTs less than 20 sec. Figures 6a-d also show the degree of transfer as estimated by the difference between the Neutral recipient group and the two other experimental groups. The

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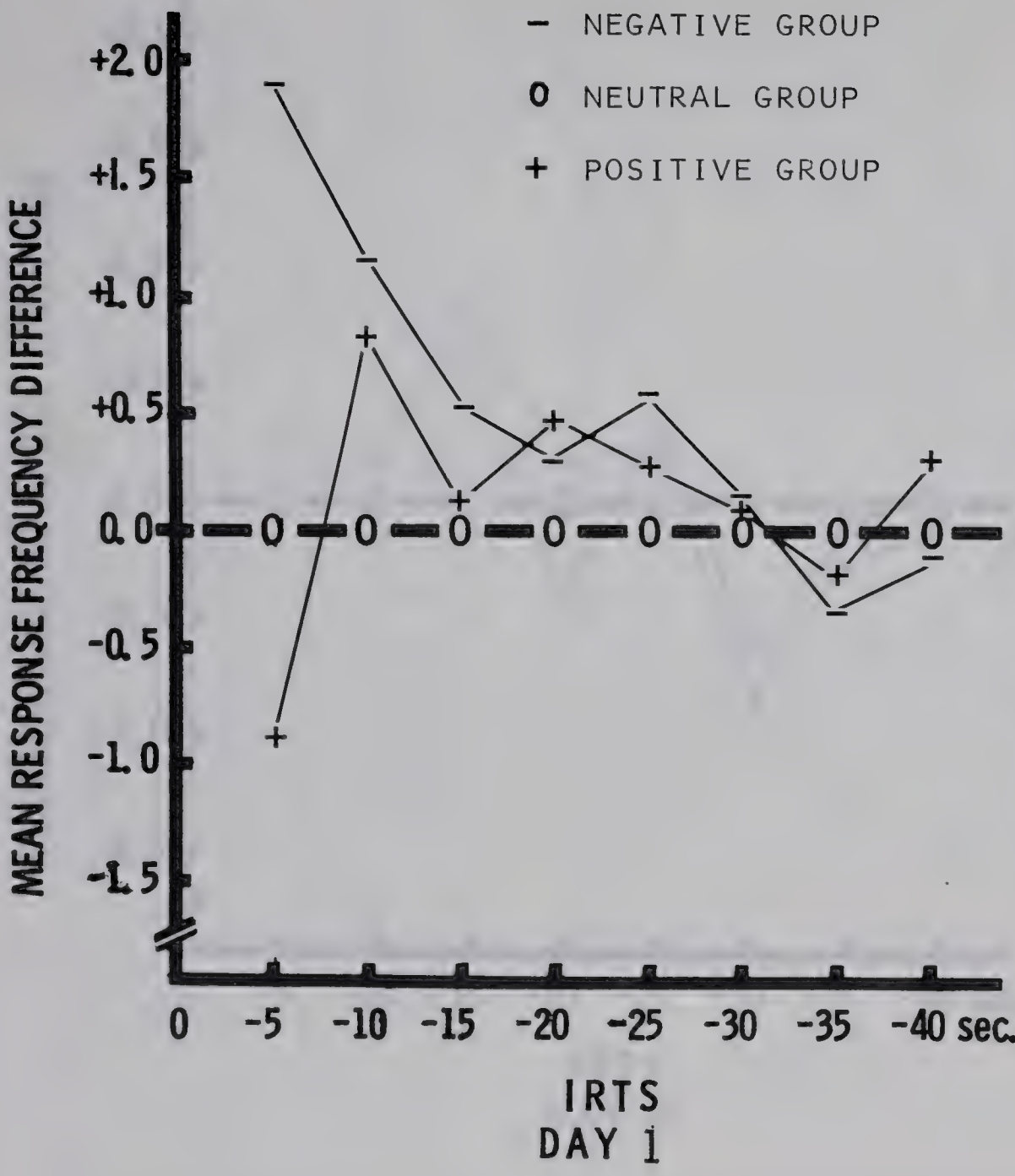
[1]Based on transformed scores.



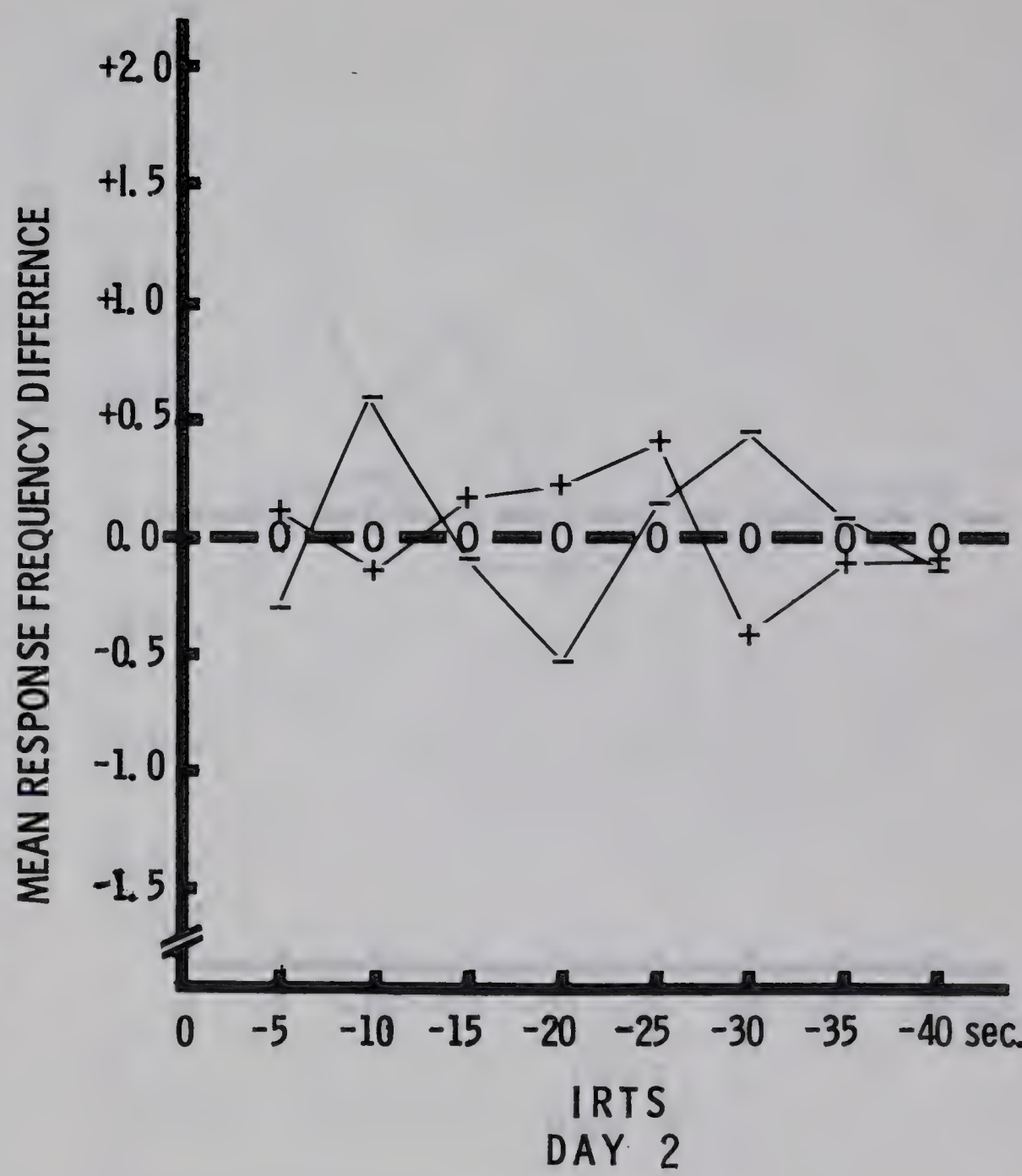
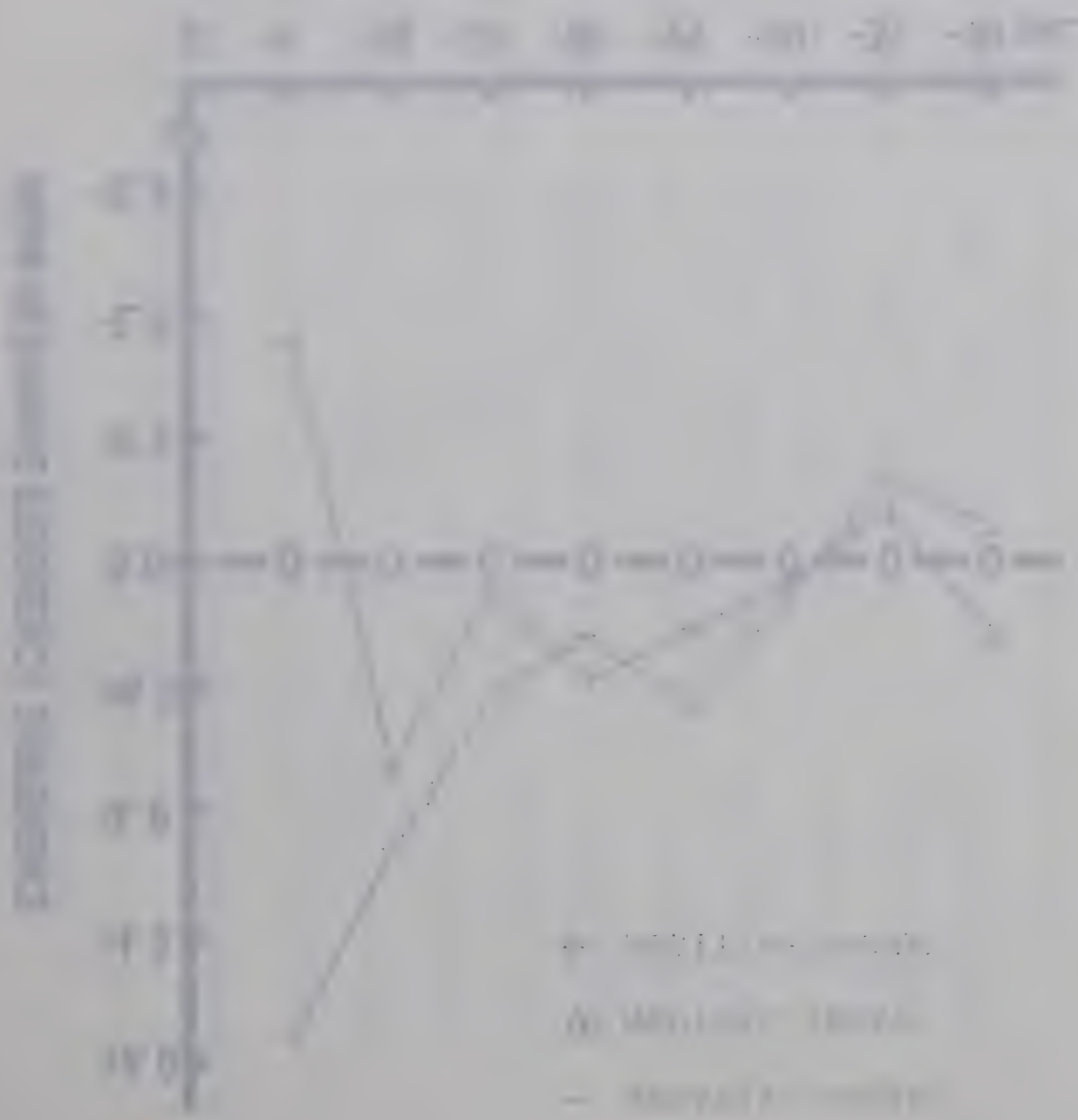
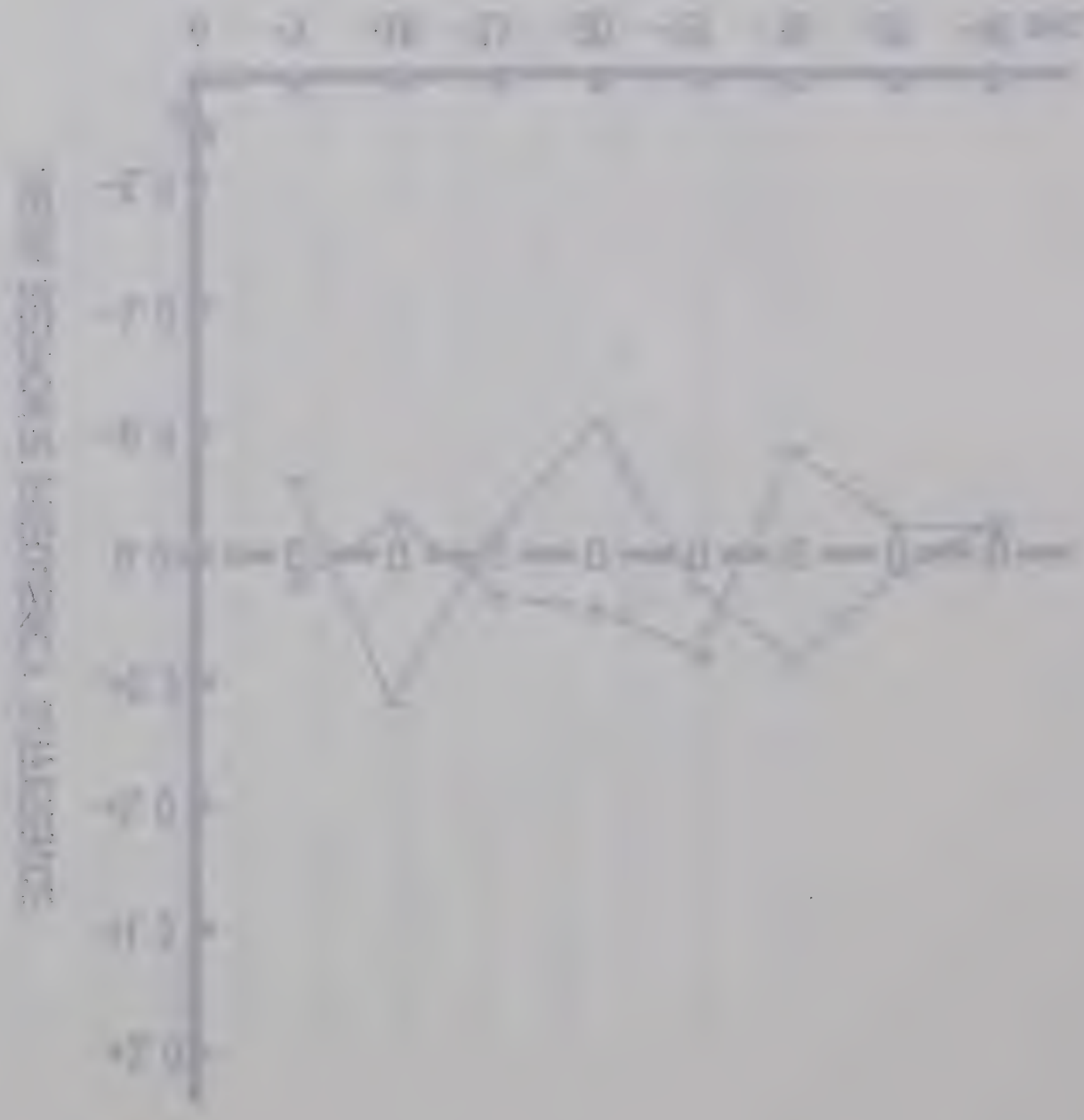
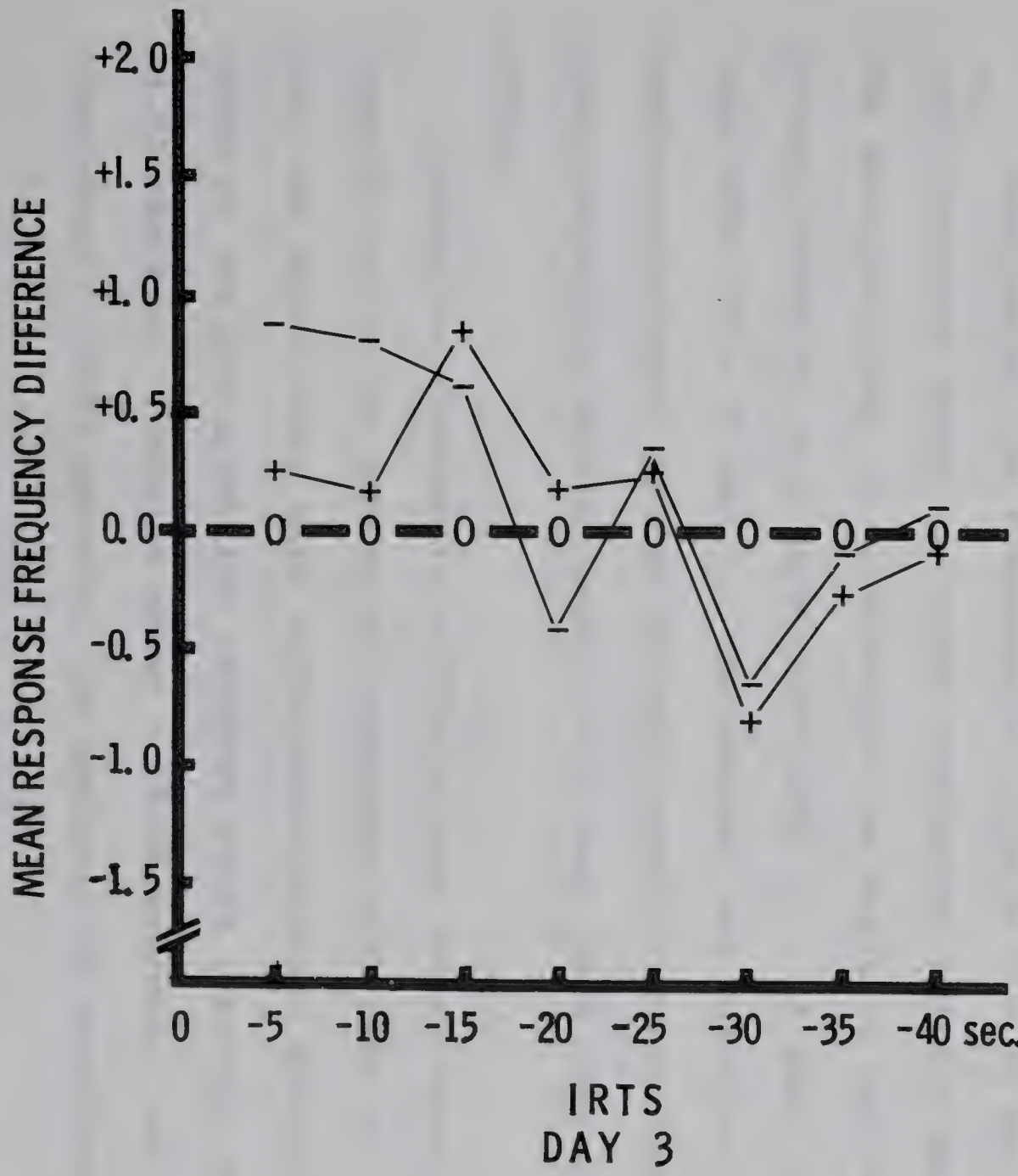


FIGURE 6A AND 6B POSITIVE AND NEGATIVE RECIPIENT GROUPS' IRTS X DAYS INTERACTIONS EXPRESSED AS DIFFERENCES FROM THE NEUTRAL RECIPIENT GROUPS' IRTS X DAYS INTERACTION -- EXPERIMENT III







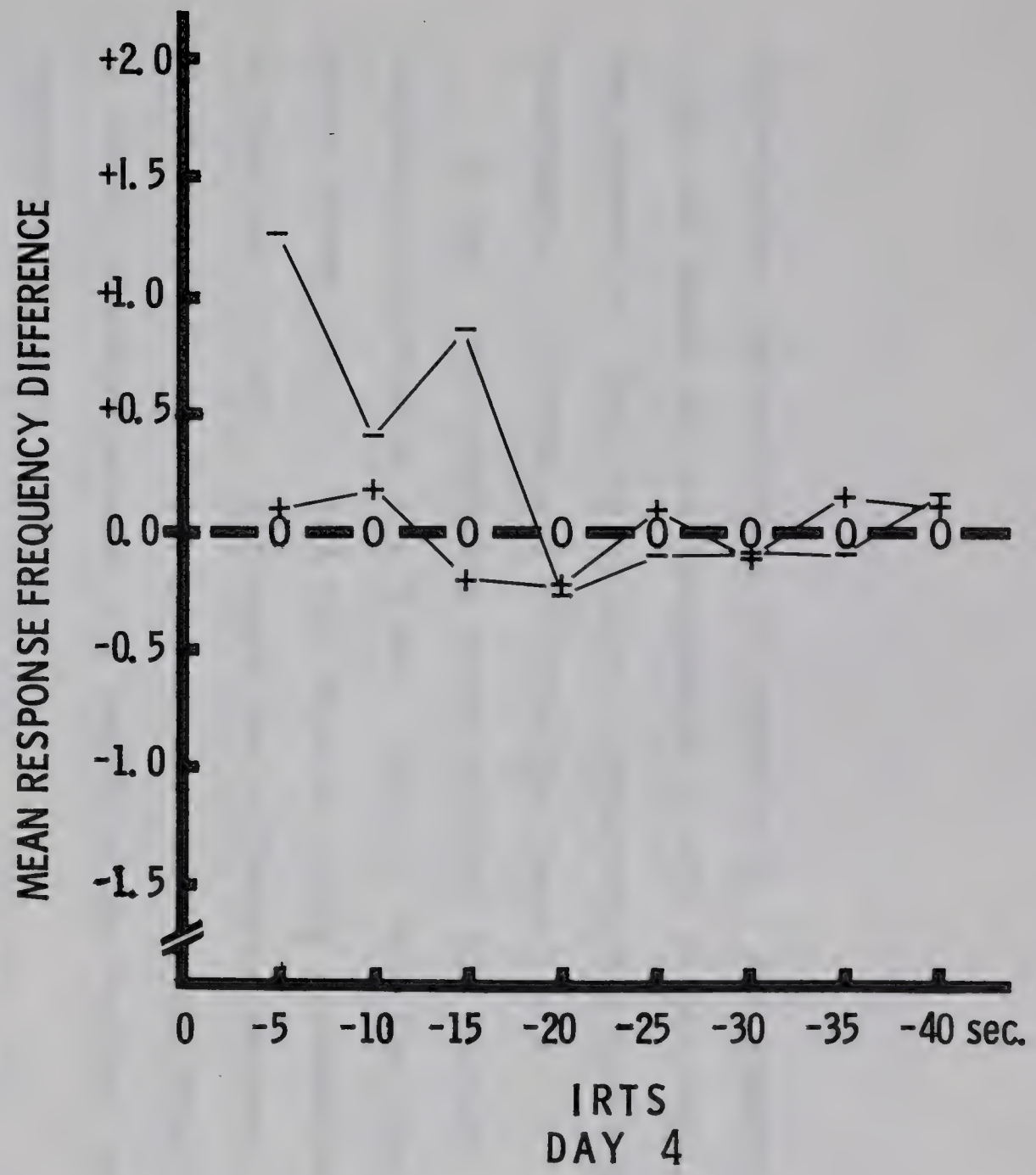
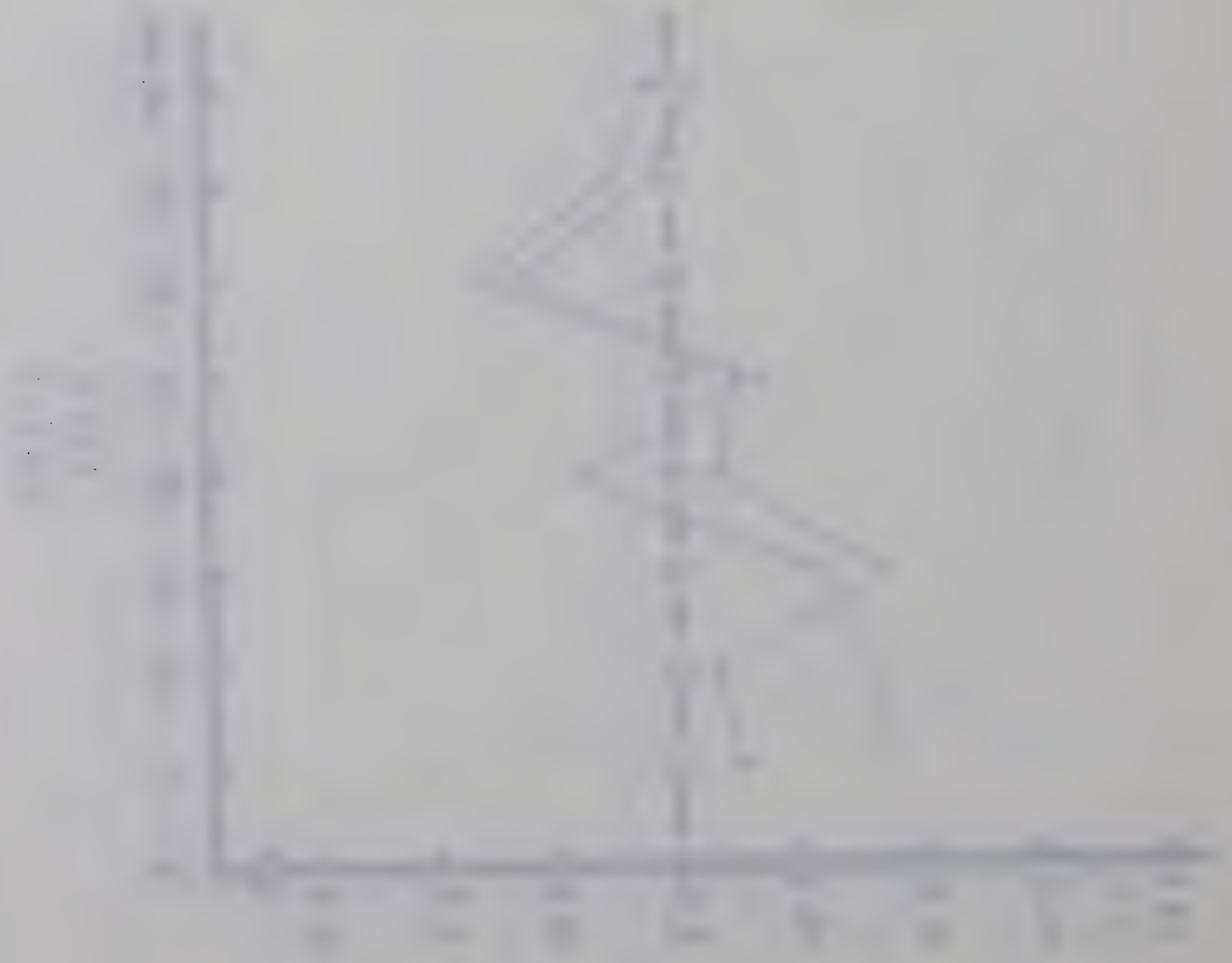


FIGURE 6C AND 6D POSITIVE AND NEGATIVE RECIPIENT GROUPS' IRTS X DAYS INTERACTIONS EXPRESSED AS DIFFERENCES FROM THE NEUTRAL RECIPIENT GROUPS' IRTS X DAYS INTERACTION -- EXPERIMENT III



differences are available in Appendix N. See Appendix O for the means of the Treatments x IRTs interaction. See Appendix P for the original untransformed means for Treatments x S^{Δ}-IRTs and S^{D}-IRTs for Days 1 - 4.

On Day 1 the Negative group yielded the highest frequency of IRTs of 0 - 5 sec. (15.57) with the Positive group lowest (12.73) and the Neutral group intermediate to the two (13.64). Although the difference between the Positive and Neutral groups was not maintained after Day 1, the difference between the Negative and Neutral groups reappeared on Days 3 and 4.

Considering the frequency of IRTs of 5 - 20 sec. for each Treatment group, a rather consistent pattern emerges. The Negative group in contrast to the Positive and Neutral groups tended to emit more short IRTs (5 - 15 sec.) and fewer long IRTs (15 - 20 sec.). The Neutral and Positive groups' performances were similar to each other; however, the Positive group generally emitted more 5 - 20 sec. IRTs than the Neutral group.

Since the Treatments x IRTs x Days interaction is largely due to the number of responses with IRTs of 0 - 5 sec. and since there were differences between groups in terms of the pre-injection response rates (Neutral group - 14.83 res/min., Positive group - 15.66 res/min., and Negative group - 15.08 res/min.) an analysis of covariance was

obtained upon the frequency of responses with IRTs of 0 - 5 sec. The results of the analysis are presented in Table 8. In no case were the within groups regression coefficients significant. The Treatments x Days interaction was significant ($P<.025$) and is presented in Figure 7. The adjusted means for each Treatment group are available in Appendix Q. The interaction indicates that on Days 1, 3, and 4 the Negative group showed a higher frequency of responses with IRTs of 0 - 5 sec. relative to the Positive and Neutral groups. Further, the Positive group emitted fewer responses with IRTs of 0 - 5 sec. than the Neutral group across all Days; the difference was particularly extreme for Day 1 after which the differences were rather small. This difference between the Positive and Neutral groups is in contrast to the results of the analysis of variance which showed that the Positive group had emitted the higher frequency of IRTs of 0 - 5 sec. for Days 2, 3, and 4.

The control and experimental data (analysis of variance) yielded the following significant ($P<.005$) main effects and interactions in common: (1) Days, (2) IRTs, and (3) Days x IRTs. The interactions are presented for the experimental and control data in Figure 8. The interactions for the two groups are of the same form and indicate that over the four days of testing Ss tended to emit fewer responses with IRTs

Table 8

Summary of Analysis of Covariance for the Three Experimental Groups' Response Frequencies in IRT 0 - 5 Sec. in Experiment III

Source of Variance	Sum of Squares	d.f.	Mean Square	F
Treatments (A)	35.76	2	17.88	
Stimulus Conditions (B)	35.03	1	35.03	4.66*
A x B	29.96	2	14.98	
Error: Pooled Subjects(C)	217.94	29	7.52	
Days (D)	2504.48	3	834.83	405.57***
D x A	35.23	6	5.87	2.85**
D x B	49.22	3	16.41	7.97***
D x A x B	11.08	6	1.85	
Error: D x C	183.20	89	2.06	
Total	3101.90	141		

*P<.050
**P<.025
***P<.005

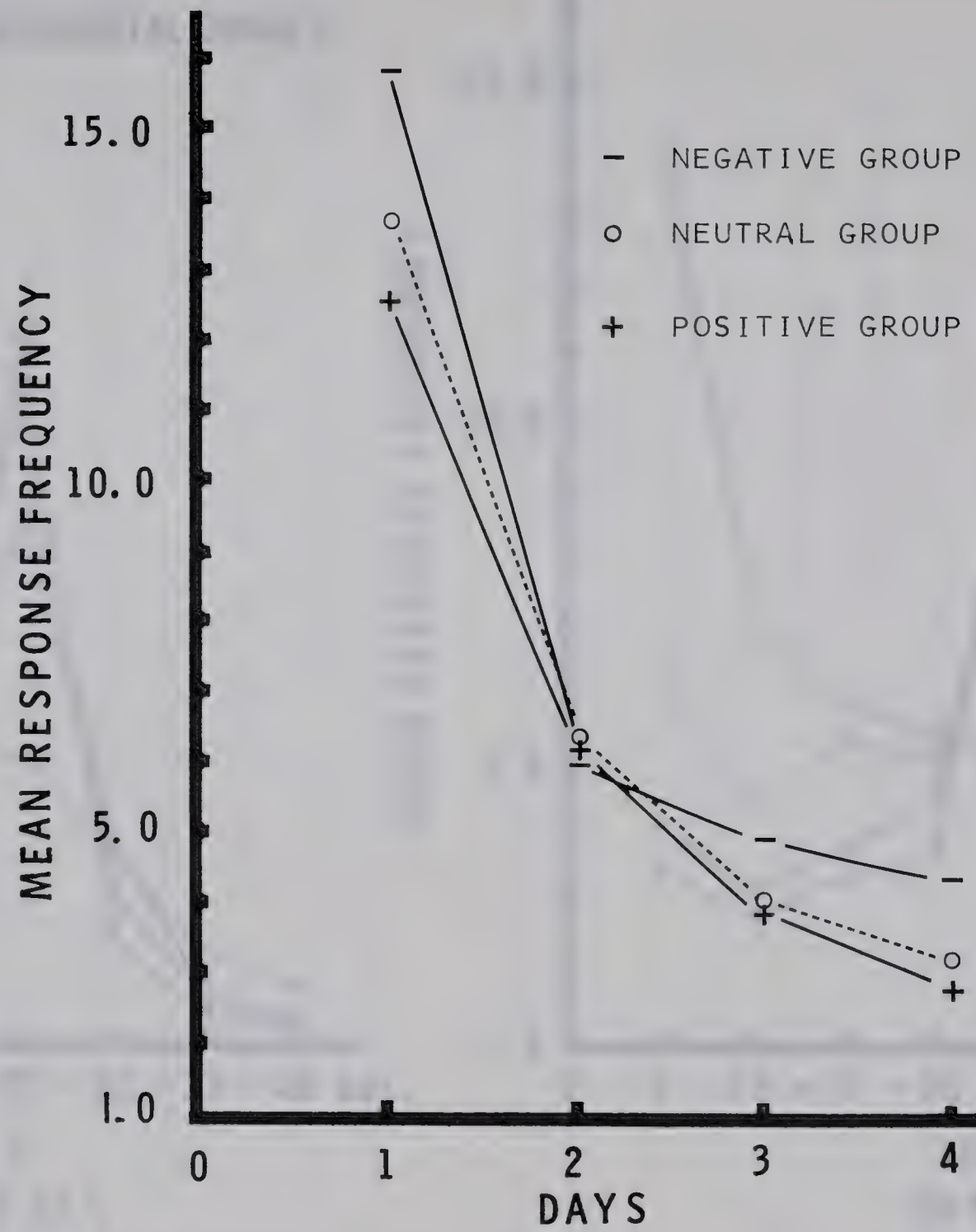
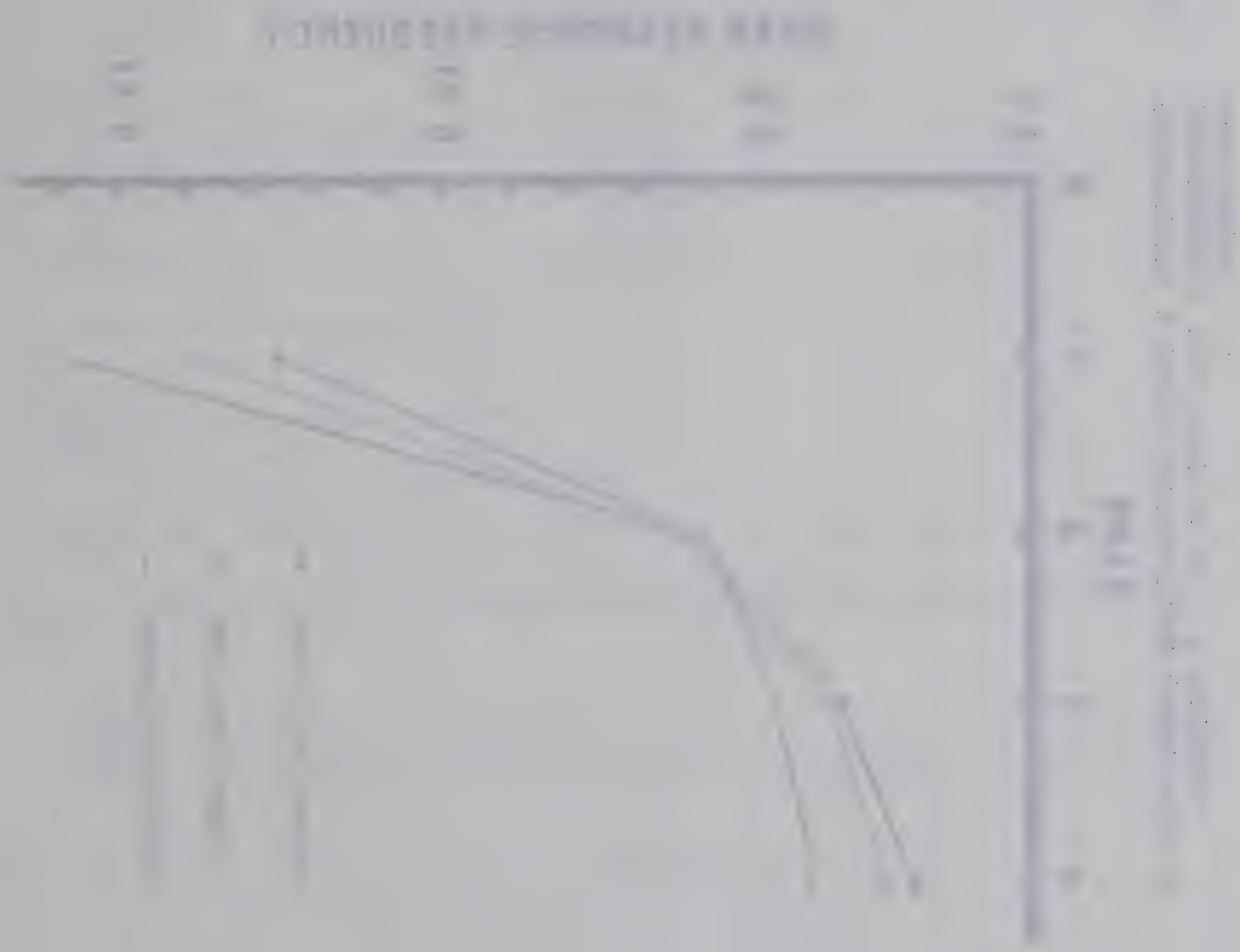


FIGURE 7 TREATMENTS X DAYS INTERACTION (ANALYSIS OF COVARIANCE) FOR IRTS OF 0 - 5 SECONDS-- EXPERIMENT III



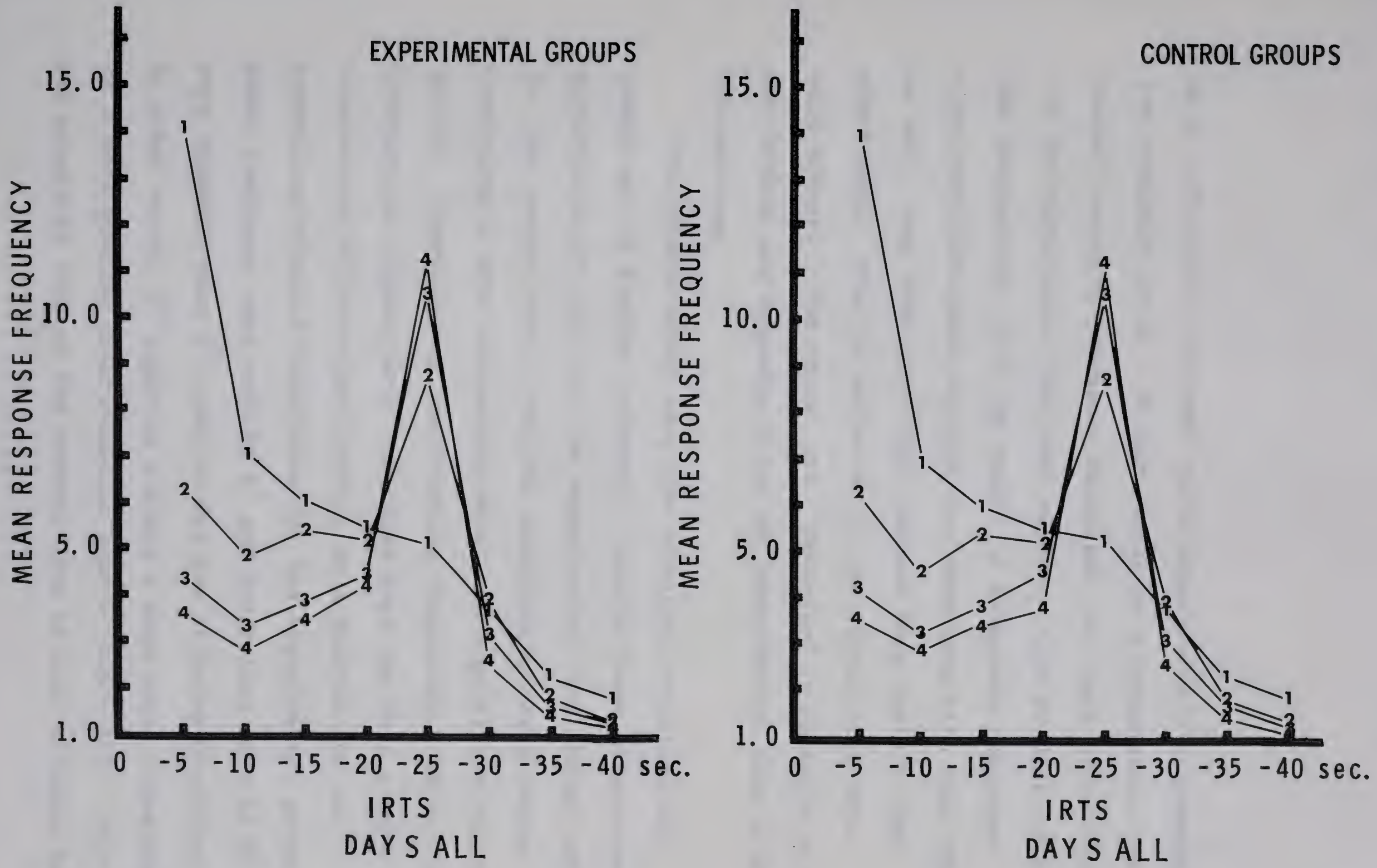


FIGURE 8 IRTS X DAYS INTERACTION FOR EXPERIMENTAL RECIPIENT GROUPS AND FOR CONTROL DONOR GROUPS--EXPERIMENT III

of 0 - 20 and 25 - 40 sec. while progressively increasing the frequency of 20 - 25 sec. IRTs; as a consequence the overall response frequency decreased over Days accounting for the significant Days main effect. Also evident from the interaction, in S^{Δ} Ss tended to eliminate responses with short IRTs more rapidly than responses with long IRTs. As well, long IRTs in S^{D} were reduced more rapidly than short IRTs. This is reflected in the significant IRTs main effect. The means are presented in Appendix R for the control and Appendix S for the experimental Days x IRTs interactions.

The effects which have the Stimulus Conditions in common are of further interest. Each of these effects was significant ($P<.005$) for the experimental data though not for the control data. The one exception is the Stimulus Conditions x IRTs interaction which was significant for both groups. Inspection of the Stimulus Conditions x IRTs x Days interaction (Figure 9a-d) indicates that the IRTs x Days interaction differs significantly as a function of the prevailing Stimulus Conditions for the experimental groups. Fewer responses were made to S^{Δ} and fewer long IRTs to S^{D} were observed when S^{D}-light-on was the Stimulus Condition. In other words, S^{D}-light-on yielded a more rapid formation of a more precise discrimination than S^{D}-light-off. This was generally true of the control data as well although the

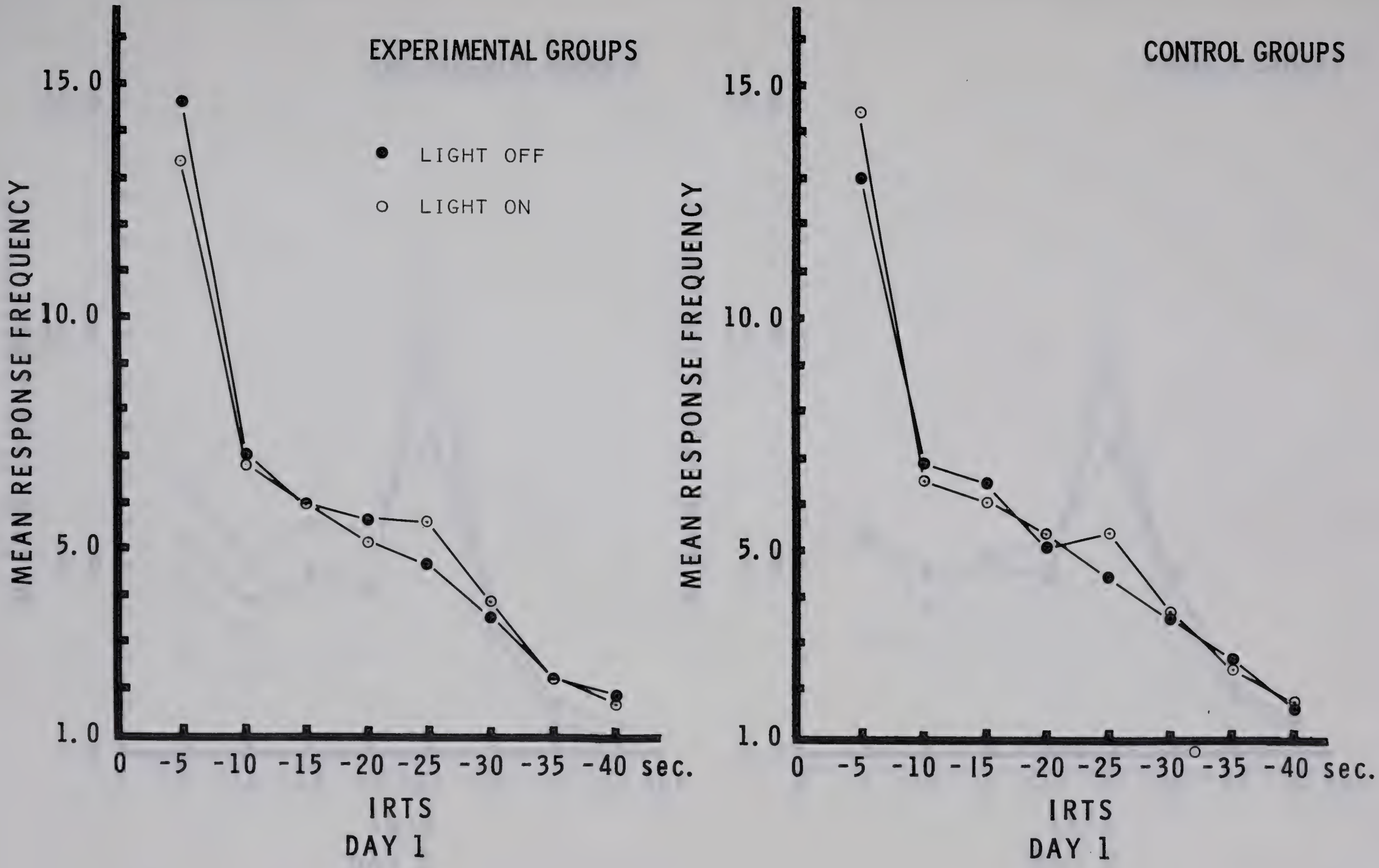
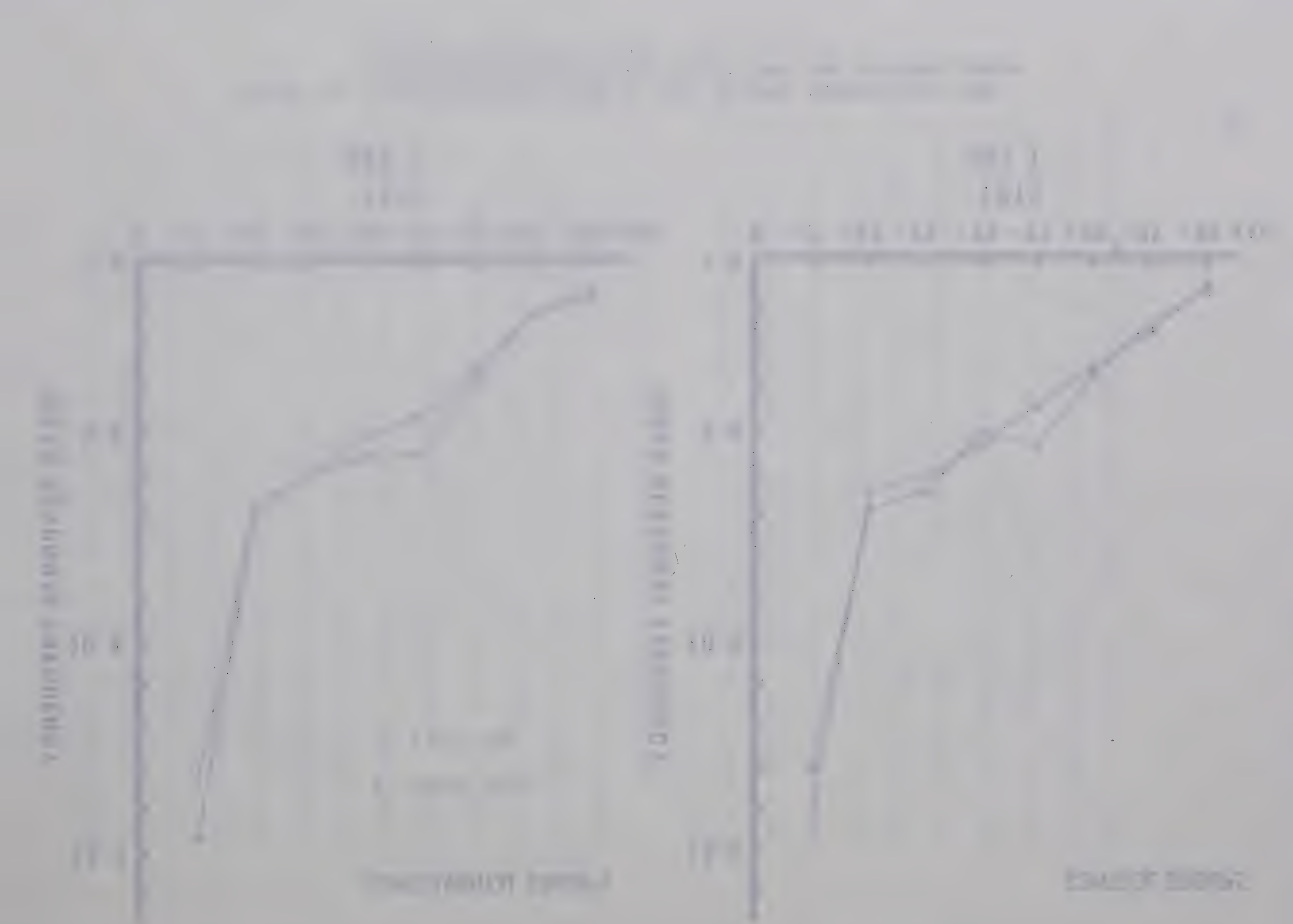


FIGURE 9A STIMULUS CONDITIONS X IRTS X DAYS INTERACTION FOR EXPERIMENTAL RECIPIENT GROUPS AND FOR CONTROL DONOR GROUPS--EXPERIMENT III



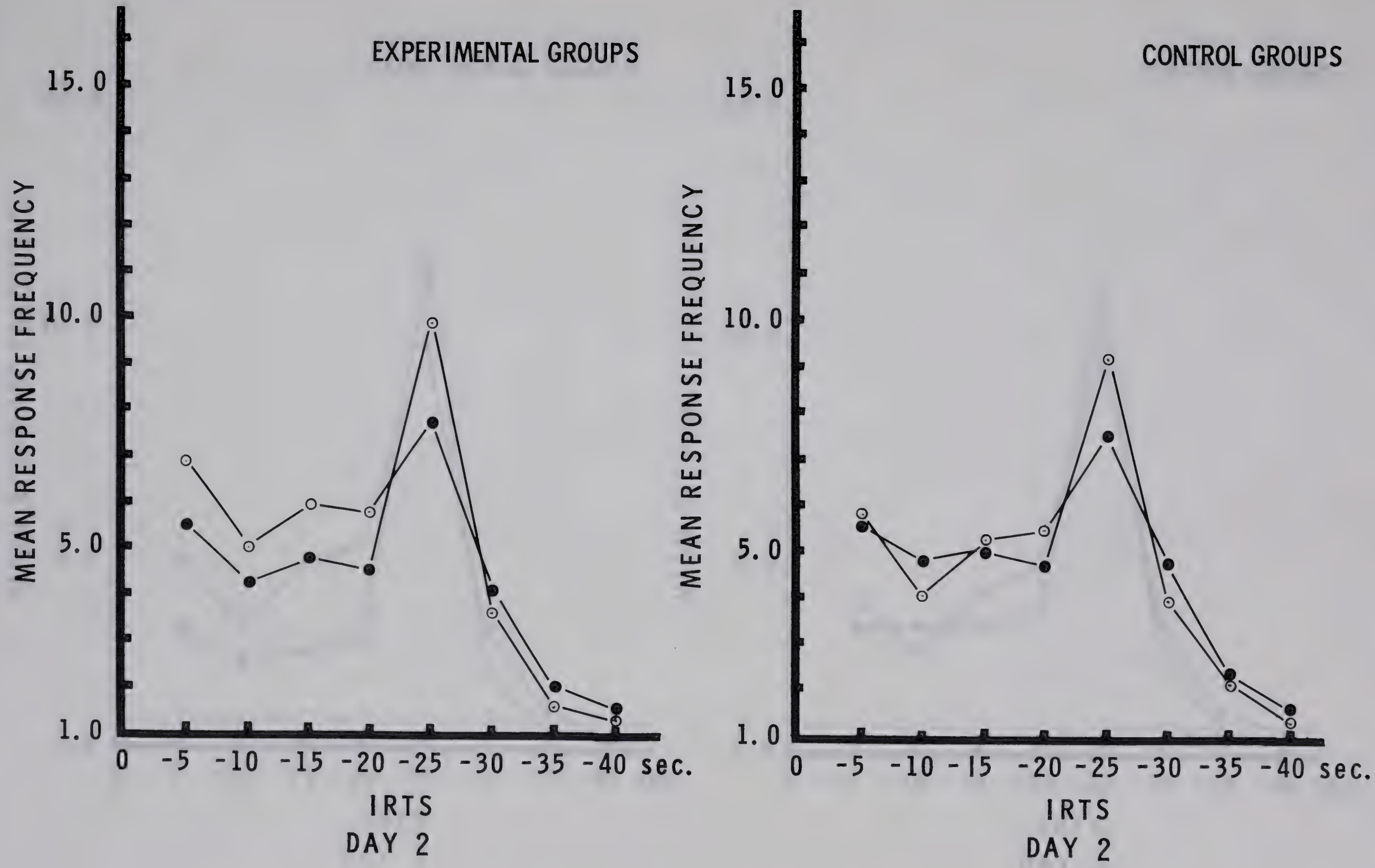
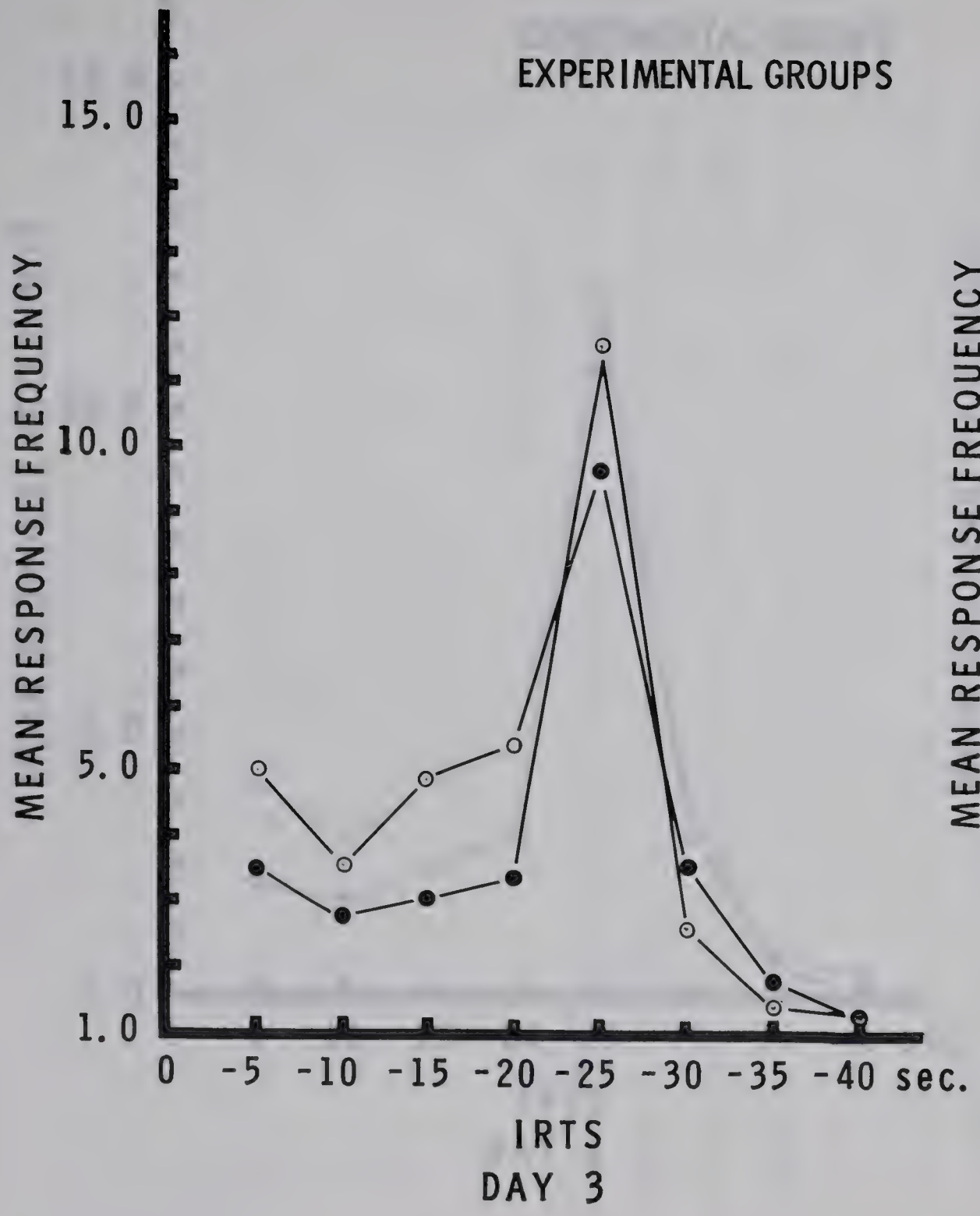


FIGURE 9B STIMULUS CONDITIONS X IRTS X DAYS INTERACTION FOR EXPERIMENTAL RECIPIENT GROUPS AND FOR CONTROL DONOR GROUPS--EXPERIMENT III



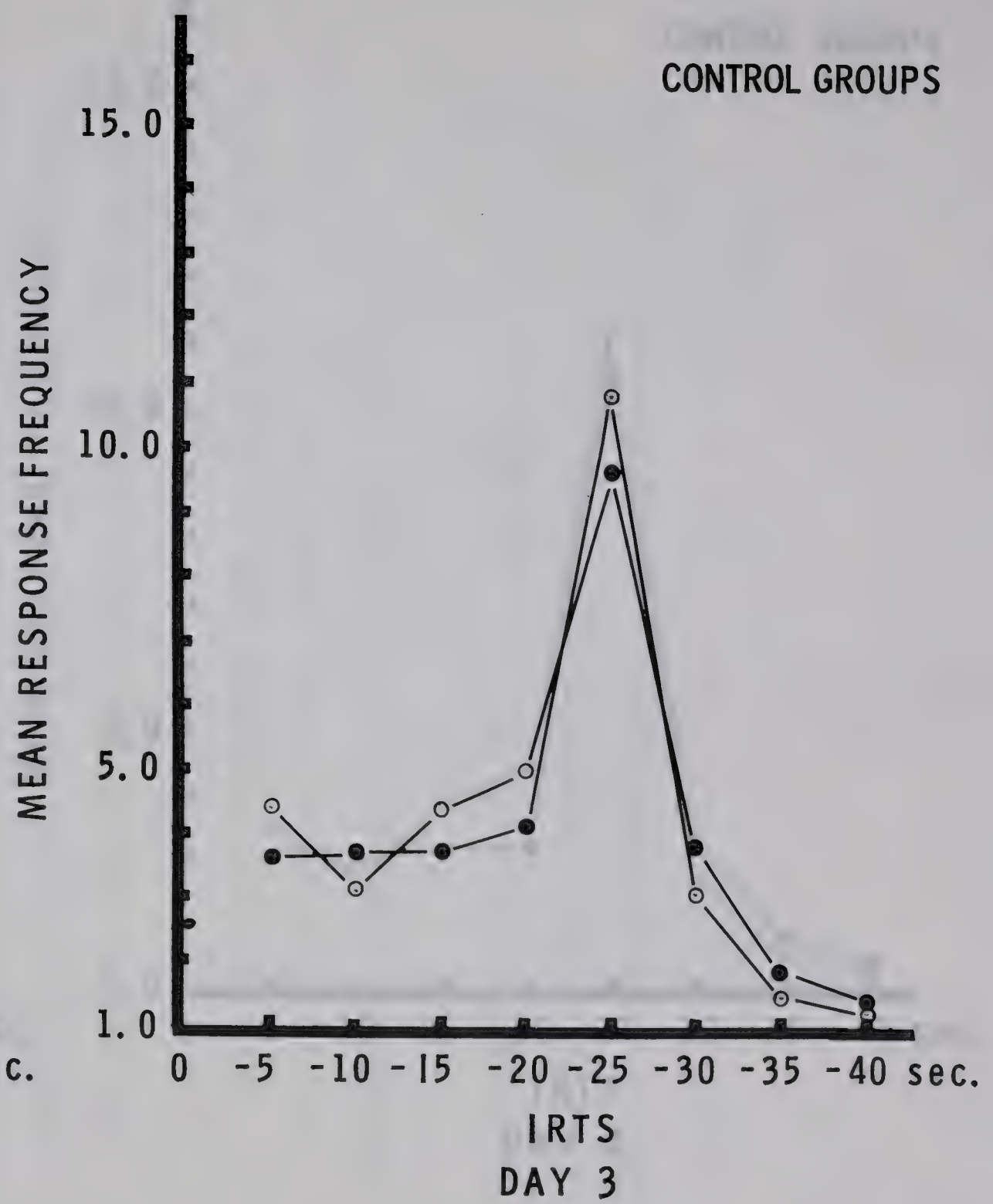
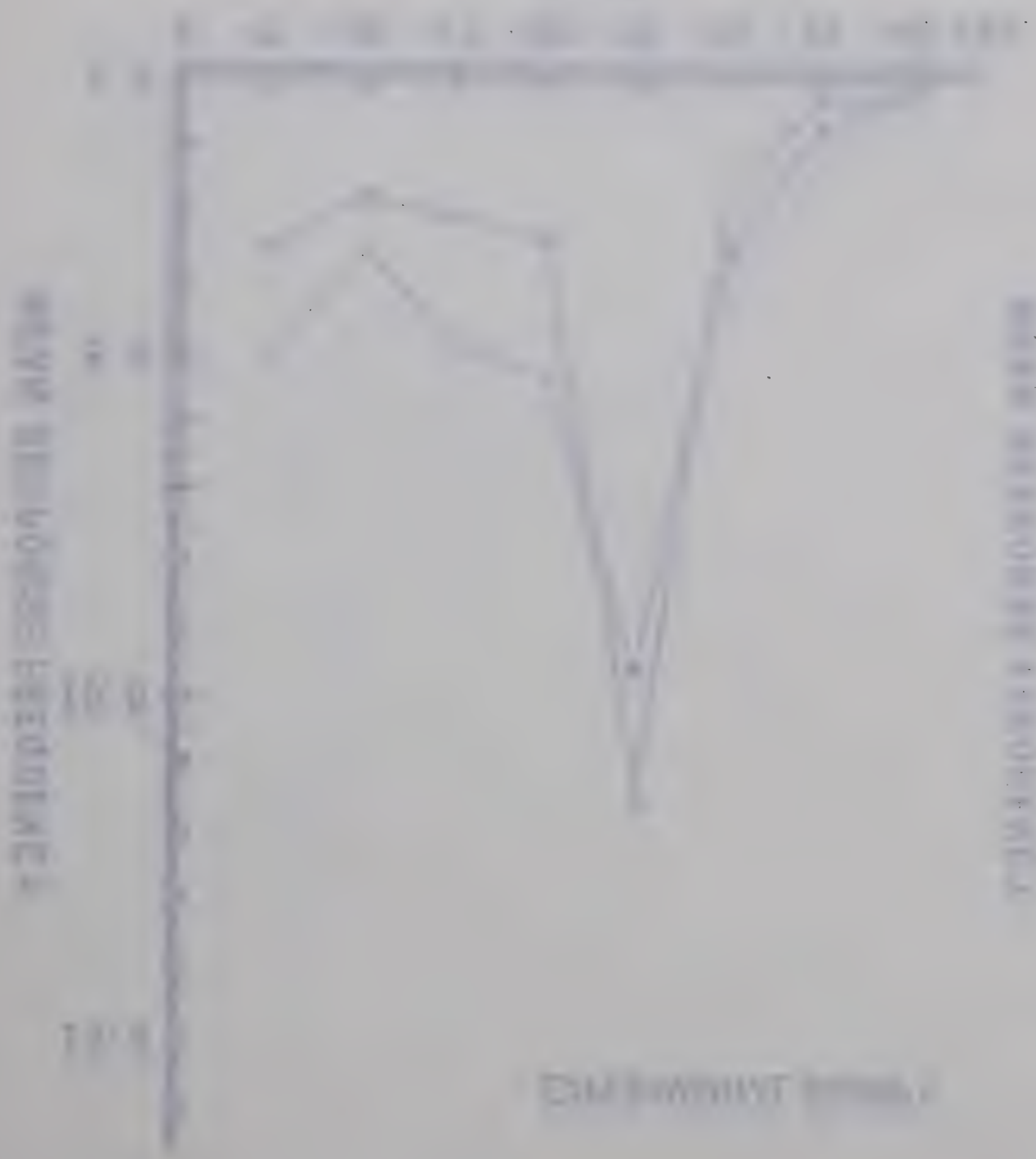
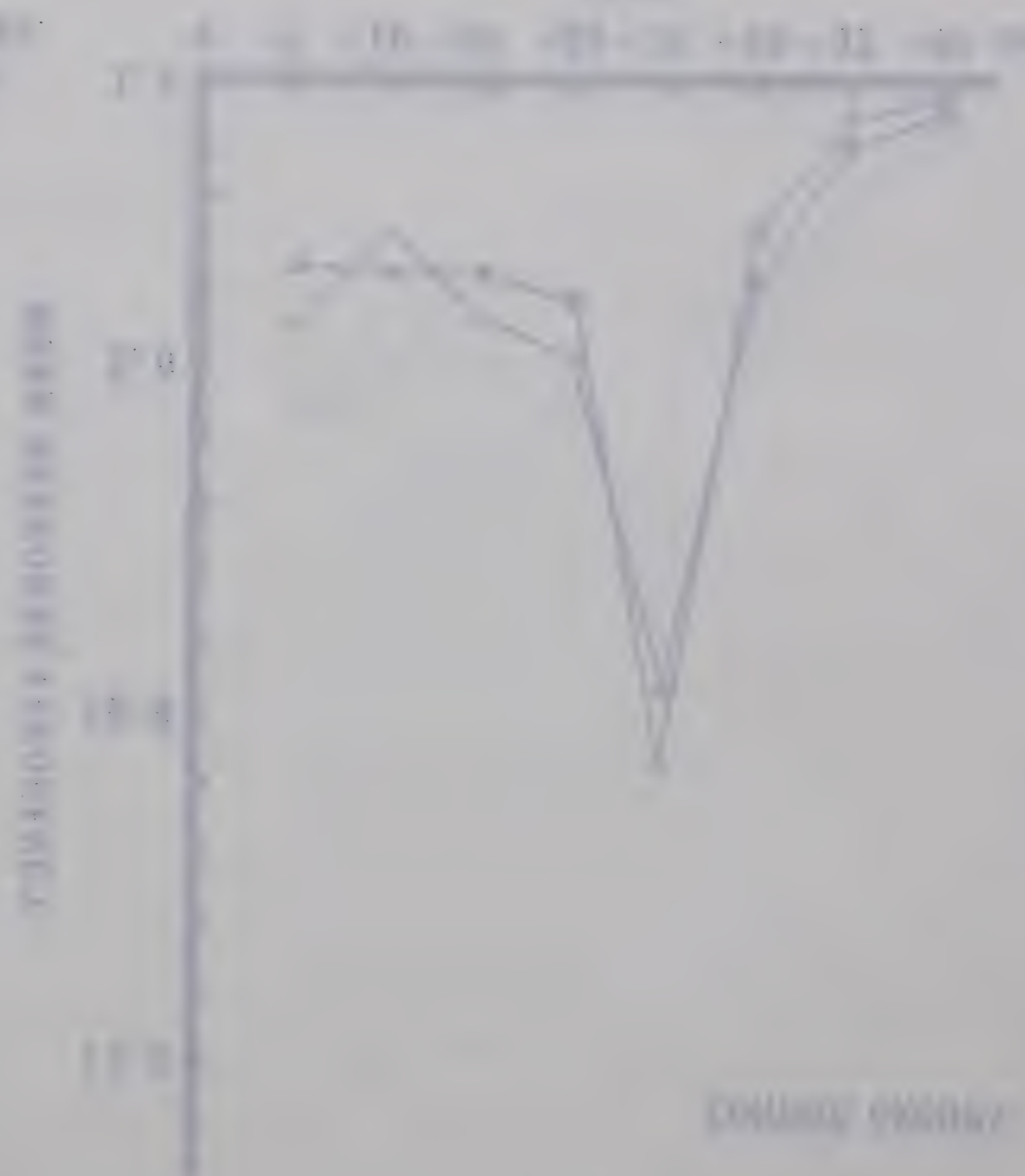


FIGURE 9C STIMULUS CONDITIONS X IRTS X DAYS INTERACTION FOR EXPERIMENTAL RECIPIENT GROUPS AND FOR CONTROL DONOR GROUPS--EXPERIMENT III





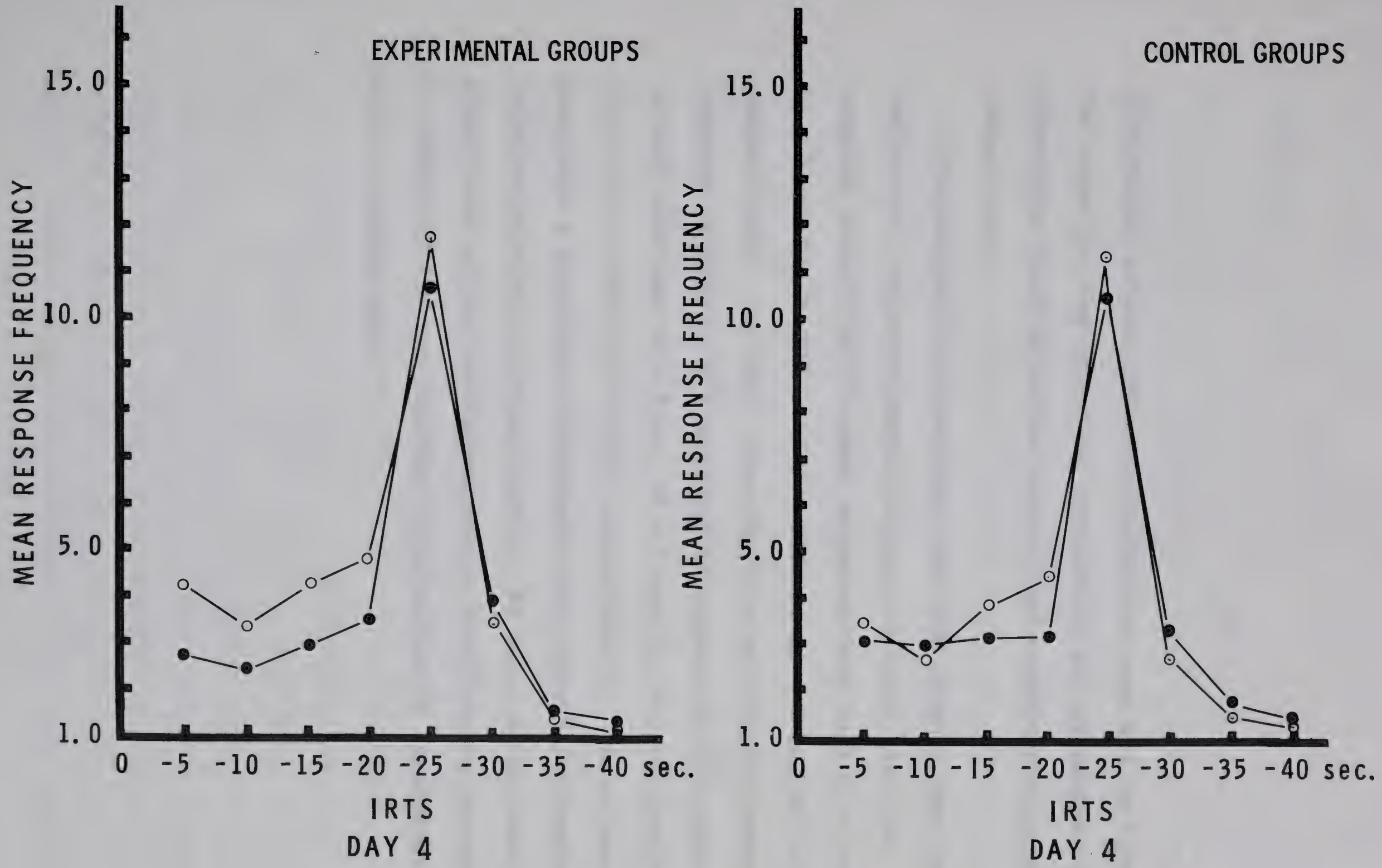


FIGURE 9D STIMULUS CONDITIONS X IRTS X DAYS INTERACTION FOR EXPERIMENTAL RECIPIENT GROUPS AND FOR CONTROL DONOR GROUPS--EXPERIMENT III

differences between Stimulus Conditions were not as large. The means for the three way interaction are presented in Appendices R and S for the control and experimental groups respectively.

The analysis of covariance upon the latency data for the control and experimental groups indicated that the response latency to S^D-onset decreased over days ($F = 56.31$, $df = 3$ and 59, $P<.005$; and $F = 140.93$, $df = 3$ and 89, $P<.005$; respectively). As well, there was a significantly greater response latency to S^D-light-off as opposed to S^D-light-on in both analyses ($F = 6.40$, $df = 1$ and 19, $P<.005$; and $F = 15.64$, $df = 1$ and 29, $P<.005$; respectively). In no case was there a significant Treatments main effect or Treatments interaction with any other factor. As well, in no case were significant within groups regression coefficients obtained. A summary of the two analyses is presented in Appendices T and U, respectively.

Discussion

Experiments I and II

The results of Experiments I and II establish that responding within the parameters of the S^D-S^Δ operant discrimination schedule employed was primarily under stimulus control once asymptotic performance was attained and when contrasting auditory or visual stimuli were used. Rats upon the attainment of asymptote tended to respond to the onset of S^D while inhibiting responses in the presence of S^Δ.

The response patterning in the presence of S^D prior to stimulus reversal was indicative of either stimulus control and/or responding to the first stimulus change following reinforcement. However, the increased proportion of responses in S^Δ with IRTs of 15 - 20 sec. reflects a slight temporal influence.

The postreversal data indicated that any temporal influence was small and that the prime and almost exclusive factor initiating responding was the stimulus condition of the environment. The Ss tended to respond almost entirely in the presence of reversed-S^Δ thus confirming the suggestion that responses during prereversal were initiated by S^D. As well, the inhibitory influence of the prereversal S^Δ upon responding is corroborated by the excessive postreversal mean response latency to reversed-S^D onset and the low post-reversal response frequency in the presence of reversed-S^D, despite the fact that these responses were reinforced. The temporal influence was characterized by the small proportion

of responses with IRTs of 15 - 25 sec.

In Experiment I variations in the auditory environment of the Ss during training and testing were difficult to control. The use of visual stimuli in Experiment II yielded more rapid acquisition of the discrimination (despite the more stringent criterion), more stable performances, and a greater degree of stimulus control of responding at asymptote. Thus, visual stimuli were more effective descriminative stimuli in this situation than auditory stimuli.

It should be noted that the evidence provided in Experiments I and II does not rule out the possibility that Ss learned something about the temporal relationship of S^{Δ} to S^{D}-onset or reinforcement. It only suggests that at asymptote Ss tended to initiate responses to S^{D} and inhibit responses in S^{Δ}. Further, the stimulus parameters of postreversal favored the demonstration of stimulus control since the temporal parameters were unaltered. Postreversal was primarily a test of whether the operant was under stimulus control or under temporal control; it did not allow for the possibility that responding may have been a function of both. To test for the degree of temporal control postreversal could have been carried out by replacing S^{D} and S^{Δ} with a single irrelevant stimulus e.g., a dim red light, but maintaining reinforcement of IRTs $\geq$ 20 sec.

Experiment III

The results of Experiment III in conjunction with the data of Experiments I and II revealed three factors which were influential in determining the response patterning of Ss injected with a brain extract: (1) The stimulus conditions employed, (2) Learning of the discriminated operant, and (3) The learning of the donors whose brain extract was injected into a recipient.

It is apparent from the control and supported by the experimental data that the response latency to S^D-light-on was shorter than the response latency to S^D-light-off. It was also evident that the response frequency in S^Δ and S^D was the highest when light-on prevailed. The decreased response latency to S^D-light-on and the increased response frequency in the presence of light was presumably due to either greater activity in the presence of light and/or because the response lever was more difficult to locate in the dark. In either case, the consequence was response inhibition in the dark and response facilitation in the light. The interaction of these properties of the stimulus conditions with reinforcement accounts for the facilitated acquisition of the discrimination for S^D-light-on as compared with S^D-light-off. The properties associated with the latter stimulus condition apparently interfered with the formation of the discriminated operant. This interaction is reflected

in the significant Stimulus Conditions main effect and the interaction of Stimulus Conditions with Days, IRTs, and Days x IRTs for the response frequency data of both the analyses of variance and covariance upon the experimental data.

The results further indicated that the experimental Ss showed a greater frequency of responses in light-on as opposed to the control Ss. This is reflected in the significance of the Stimulus Conditions x IRTs x Days interaction for the experimental group; an interaction which was not significant for the control groups (Fig. 9). This implies a possible sensitization of the brain extract injected Ss to light-on. However, the form of the interactions were similar, the differences between the two groups were small, and the fact that the control data was not significant may be attributed to the smaller degrees of freedom associated with the F tests. Also, contrary to a sensitization interpretation of the experimental data is the fact that on Day 1 the experimental groups emitted a higher frequency of responses in both S^{Δ}-light-off and S^{D}-light-on. This is in contrast to their subsequent performance on Days 2 - 4 (Fig. 9). It might be mentioned, the control and experimental groups' performances were not independent as illustrated by the correlation data; a comparison of the results of each group, therefore, is not justified. However, in light of the

evidence reported by Ungar (1966b) and Røigaard-Petersen et al. (1966) a sensitization to light factor may have relevance for an interpretation of their results since light and dark were used as discriminative stimuli. Both investigators report that recipients tended to respond more to the opposite stimulus conditions to which the donors had been reinforced.

Learning of the discriminated operant was a relevant factor in influencing the response frequency and response latency to S^D-onset over Days and also in relation to the frequency of IRTs over Days. The data of Experiments I and II and the Days x IRTs interaction (donor control groups) of Experiment III suggest that Ss learned two things during the formation of the discrimination. First, Ss apparently learned to inhibit responses to S^Δ and respond to S^D-onset. This is reflected in the Days x IRTs interaction of Experiment III in which the formation of the discrimination is characterized as a shift of short and long IRTs to IRTs of 20 - 25 sec. over Days. A reduction of the overall response frequency and a reduction of the response latency to S^D-onset is a consequence of this shift. Thus, it is the formation of the discriminated operant which is reflected in the significant Days main effects on the frequency and latency data for both the control and experimental groups. Secondly, Ss apparently learned something about the temporal relationship of IRTs in

the presence of S^{Δ} to potential reinforcement and/or S^{D}-onset. Evidence for this is seen in the Days x IRTs interaction of the control data for Experiment III (Fig. 8). Responding in the presence of S^{Δ} was inhibited more readily for shorter IRTs than for longer IRTs. These points are characteristic of what might be termed S^{Δ}-IRT inhibitory and S^{D}-excitatory gradients about IRT 20 sec. As a function of reinforcement, inhibition and excitation of responding is strengthened and the boundaries of the gradients become reduced. Thus, within any one session Ss demonstrate a decreased probability of short IRTs i.e., differential inhibition, in the presence of S^{Δ} and an increased probability of short IRTs i.e., differential excitation, in the presence of S^{D}.

Unexplainable by either the Simulus Conditions or learning factors are the differences obtained between the brain extract injected groups over IRTs and Days. The differences between groups for IRTs 0 - 20 sec. are in accordance with the transfer hypothesis. Across all Days and IRTs the greatest differences between Treatments were obtained for IRTs of 0 - 5 sec. on Day 1. Considering the Neutral recipient group as the norm, on Day 1 the Positive group demonstrated an inhibition of short IRTs in the presence of their donor's S^{Δ} (Figures 5, 6 & 7). In contrast, the Negative recipient group showed a facilitation of short IRTs in the presence of their donor's S^{D}. This corresponds with the hypothesis that specific stimulus control of responding was transferred from the donors

to the recipients. That is, differential performances were demonstrated by the recipient groups as a function of whether their donors had been reinforced or not reinforced for short IRTs in the presence of the recipient's S^{Δ}. The analysis of covariance upon the IRTs of 0 - 5 sec. (Fig. 7) ruled out the possibility that the differences between groups was a function of differential response rates prior to testing.

With the exception of Day 2, the Negative recipient group maintained its high frequency of IRTs of 0 - 5 sec. through Day 4. Actually, the difference between the Negative group and the other groups tended to increase from Day 2 to Day 4. This slow extinction of short IRTs was maintained despite the fact that the Negative recipient Ss emitted considerably more nonreinforced responses with IRTs of 0 - 5 sec. (331.5, S's mean) than the Positive (239.7) or Neutral (257.4) recipient Ss over the four days of testing. It would appear, therefore, that the information transferred to the Negative recipient group was potent enough to maintain the deviant response tendency despite continuous nonreinforcement of such responses.

Following Day 1, the Positive and Neutral groups' performances were approximately the same; however, it is noteworthy that over all days of testing the Positive group maintained a lower frequency of 0 - 5 sec. IRTs (Fig. 7; analysis of covariance). This consistent pattern suggests

that the Positive group learned to inhibit short IRTs in the presence of S^{Δ} more rapidly and with fewer nonreinforced responses than the Neutral group. That the difference between the Positive and Neutral groups was not more extreme may be because the Positive recipient group's performance reflected a maximum rate of extinction. Also, the donors at asymptote demonstrated some responses in the presence of S^{Δ} with IRTs of 0 - 5 sec. Therefore, the probability of such responses was not zero. Such a response tendency may have been transferred to the Positive recipients thereby confounding the extinction of IRTs of 0 - 5 sec. This point is elaborated upon below in relation to the frequency of IRTs of 5 - 20 sec. for the Positive recipients.

The Negative group, in comparison with the Neutral group showed facilitation of responding to S^{Δ} (IRTs of 0 - 15 sec.). Shorter IRTs in the presence of S^{Δ} were emitted more than longer IRTs both within and between days. Thus, it is apparent that the performance of the Negative recipients in the presence of S^{Δ} reflected their donors' pattern of responding to S^{D} i.e., the donors' S^{D}-excitation gradient. Thus, the learning of the donors in the presence of S^{D} was apparently influential in determining the performance and subsequent learning of the Negative recipients to S^{Δ}. In this case, the information transferred was detrimental to the formation of the discriminated operant in the sense that inhibition of short IRTs in S^{Δ} was less rapid than for the

Neutral group. On the other hand, the transfer was beneficial in the respect that longer IRTs were inhibited more rapidly than for the Neutral group.

The Positive and Neutral recipient groups showed a facilitation of responding for 5 - 20 sec. IRTs (Figs. 5 & 6) and an inhibition of responding for 0 - 5 sec. IRTs. However, it is evident from Figure 6 that the Positive group had a greater tendency to emit long IRTs and inhibit short IRTs in the presence of S^{Δ} than the Neutral group; this is especially evident for Days 1 - 3. It is apparent that the performance of the Positive recipients in S^{Δ} reflected the donors' pattern of responding in S^{Δ} i.e., the donors' S^{Δ}-inhibition gradient. The results of the analysis of variance show that the Positive recipient's response frequency for long IRTs in S^{Δ} was higher than the Neutral recipient's; thus, it would appear that the transferred S^{Δ}-inhibition gradient had a cumulative effect upon the recipient's long IRTs. Again, the learning of the donor was apparently influential in determining the performance and subsequent learning of the recipient. The information transferred was detrimental to the formation of the discriminated operant to the extent that in the presence of S^{Δ} inhibition of long IRTs was less rapid than for short IRTs. However, the information was beneficial to the extent that short IRTs were readily inhibited.

The Treatments x Days x IRTs interaction revealed no consistent differences between groups in the presence of S^D. This is presumably because the behavioral measure was less sensitive for long IRTs i.e., IRTs $\bar{>}$ 20 sec.; only one response in S^D was reinforced at a time. The maximum number of responses becomes more and more limited with longer and longer IRTs e.g., the maximum frequency of IRTs of 5 sec. is 720 while the maximum frequency of IRTs of 25 sec. is 144 for a single session. Also, the fact that long IRTs in S^Δ were facilitated for the Positive group and inhibited of the Negative group while short IRTs in S^Δ were facilitated for the Negative group and inhibited for the Positive group would tend to balance out so that both groups would experience S^D-onset approximately the same number of times. Indeed this was the case, Ss of the Negative group for each of the four days of testing experienced S^D-onset an average of 100.3 times a day while Ss of the Positive group experienced S^D-onset 99.7 times. On the other hand, Ss of the Negative group emitted a daily average of 155.6 responses in the presence of S^Δ while Ss of the Positive group emitted an average of 130.6 responses on each of the four days of testing.

The correlation data are also consistent with the hypothesis that the performance of the donor was influential

in determining the performance of the recipient. The recipient's performance in the presence of light-on or light-off tended to be negatively correlated or uncorrelated with its donor's performance in the opposite stimulus condition. On the other hand, the performance of the recipients was positively correlated with their donors' performance in the same stimulus condition. The lower correlations for the Negative recipient group may have been due to the influence of reinforcement which for the Negative group would tend to interfere with the transfer effects. Although it seems reasonable to suppose that reinforcement might attenuate the correlations for the Negative group, it is not entirely clear how such a mechanism would operate.

The latency correlations for the Positive recipient group, while not significant, suggested that recipient Ss tended to have short or long latencies to S^D-onset in correspondence with their donor's latency to the same stimulus condition. That the Negative group did not show consistent negative correlations with their donors may again be attributed to the interaction of reinforcement with the transfer factor thereby reducing correlations.

The author is led to conclude that the effects demonstrated within Experiment III are in support of the hypothesis that an information transfer was effected

interorganismically from a trained S to an untrained S. The effects were a function of the stimulus conditions employed; the recipient groups' response patterns in the presence of a particular stimulus reflected the same general pattern which its donor group had demonstrated. The data are interpreted as support for a biochemical interpretation of information storage.

The behavioral character of the information transferred is consistent with both a specific S-R paradigm and an incentive motivation interpretation. That is, whether the information transferred tended to facilitate/or inhibit either the initiation of a specific response or the initiation of a motivational process to a particular stimulus cannot be discerned from the data. However, both positions are equally tenable and deserve further investigation and clarification.

Methodological Considerations

Several possible objections may be expressed with the evidence presented in Experiment III and also with other experiments which have apparently demonstrated an interorganismic information transfer. The data may be an artifact of differential treatments inadvertently administered to the experimental treatment groups. A double blind technique was not employed for training, injecting, or testing the recipients. However, what appeared to be satisfactory precautions to E were taken in all instances so that identifications of

an S's Treatment group was not known during injection or the initiation of any experimental session. Criticism of the Experiment on this basis can only be waived, however, by replication of the results using a double blind technique.

The choice of IRT frequencies as a behavioral measure in this study was necessary because of the decision to test the recipients on the same schedule as the donors had been trained. However, an evaluation of whether a transfer of information had been effected would have been easier if the recipients were tested on a fixed interval S^D-S^Δ discrimination schedule. In this situation an analysis of a recipient's frequency of responses in S^D and S^Δ would have been a sufficient measure.

No attempt was made to identify the transfer factor involved. However, centrifuging of the brain homogenate was done at 37,800 x g at R max for 30 minutes. LaBella, Reiffenstein, and Beaulieu (1963) using bovine posterior pituitary glands homogenized in sucrose and centrifuged at 20,200 x g at R max for 15 minutes removed nuclei and debris, mitochondria, and heavy microsomes from the homogenate. They report that the remaining microsomal and supernatant fractions contained approximately 61-65% of all protein and 49-55% of the total RNA. Thus, it may be concluded that the extracts prepared in Experiment III contained significant concentrations of both protein and RNA.

Research which attempts to effect an information transfer

interorganismically raises several methodological as well as theoretical problems which need clarification. A general list of these problems is presented below and is not presumed to be exhaustive. First, assuming a transfer of information does take place:

1. What is the chemical nature of the transfer factor?
2. How does the factor make its way to the brain? and by what mechanism does it become active in directing behavior?
3. Can the transfer of information interorganismically be effected in organisms other than the rat?
4. Are both classically and instrumentally conditioned responses amenable to transfer?
5. What is the latency following injection of the recipient before the information tranferred becomes active?
6. What is the nature of the information transferred? That is, is it primarily motivational or does it involve specific response tendencies? or both?
7. Are genetic factors significant variables in facilitating or inhibiting an information transfer?
8. Is the information transferred specific only to the task learned by the donor or can the information be generalized to associated tasks?

Second, assuming that an information transfer does not take place:

1. What are the relevant variables which have been influential in effecting the apparent behavioral changes in organisms following the injection of a brain extract?

The above points and questions illuminate the rationale for continued research and dissemination of information related to the demonstration of an interorganismic information transfer.

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APPENDIX

Appendix A

Cumulative Response Frequencies and Proportions for Each Five Second IRT During Prereversal (N = 7) -- Experiment I

IRT:	0-5	-10	-15	-20	-25	-30	-35	-40	>40	Σ sec.
Frequency	35	17	30	100	389	28	7	2	26	634
Proportion	05.5	02.7	04.7	15.8	61.4	04.4	01.1	00.0	04.1	99.7%

Appendix B

Cumulative Response Frequencies and Proportions for Each Five Second IRT During Postreversal (N = 7) -- Experiment I

IRT:	0-5	-10	-15	-20	-25	-30	-35	-40	>40 sec.	Σ
Frequency	420	49	78	85	46	17	9	7	48	759
Proportion	55.3	06.5	10.3	11.2	06.1	02.2	01.2	01.0	06.3	1.001%

Appendix C

Cumulative Response Frequencies and Proportions for Each Five Second IRT During Prereversal (N = 8) -- Experiment II

IRT:	0-5	-10	-15	-20	-25	-30	-35	-40	>40 sec.	Σ
Frequency	31	10	12	26	514	48	12	2	0	655
Proportion	04.7	01.5	01.8	04.0	78.5	07.3	01.8	00.0	00.0	99.6%

Appendix D

Cumulative Response Frequencies and Proportions for Each Five Second IRT During Postreversal (N = 8) -- Experiment II

IRT:	0-5	-10	-15	-20	-25	-30	-35	-40	>40 sec.	Σ
Frequency	344	94	53	56	26	19	13	7	20	632
Proportion	54.4	14.9	08.4	08.9	04.1	04.0	02.1	01.1	03.2	100.1%

Appendix E

Discrimination and Latency Data During Acquisition and Testing for Experiments I and II

Parameter	Experiment I	Experiment II
Acquisition		
Days to Criterion[1]		
(Mean)	10.7 days	5.5 days
(Range)	7-14 days	4-8 days
(S.D.)	2.9	1.2 days
Day to Day DI Variation[2]		
(Mean)	12.4%	07.8%
(Range)	00-43%	00-25%
(S.D.)	15%	06%
Testing		
Prereversal		
DI		
(Mean)	71.2%	87.9%
(Range)	60-89%	83-99%
(S.D.)	10%	05%
LI		
(Mean)	1.98 sec/res.	2.38 sec/res.
(Range)	0.81-3.12 sec/res.	1.64-3.92 sec/res.
(S.D.)	0.80 sec/res.	0.73 sec/res.
Postreversal		
DI		
(Mean)	16.7%	13.4%
(Range)	04-37%	04-25%
(S.D.)	12%	08%
LI		
(Mean)	50.06 sec/res.	102.63 sec/res.
(Range)	11.59-324.20 sec/res.	35.80-1743.20 sec/res.
(S.D.)	115.00 sec/res.	874.74 sec/res.

[1] Criterion for asymptotic performance was set at DI = 70% x three consecutive days for Experiment I and DI = 80% x three consecutive days for Experiment II

[2] Refers to day to day changes in the DI for all Ss after the 2nd day of S^D-S^Δ training until criterion was met.

Appendix F

Summary of the Sources of Variance and Their Respective Levels -- Experiment III

Source of Variance (df)

A - Treatments (5)

Donors
- A_1 - Noninjected donors
- A_2 - Saline injected donors

Recipients
- A_3 - Positive
- A_4 - Neutral
- A_5 - Negative

B - S^D Stimulus Conditions (2)

- B_1 - Light-on
- B_2 - Light-off

C - Subjects (6)

C_1 -
C_2
C_3 Ss pooled within A and B
C_4
C_5
C_6 -

D - Days (4)

- D_1 - Day 1
- D_2 - Day 2
- D_3 - Day 3
- D_4 - Day 4

F - IRTs (8)

- F_1 - IRT 0 - 5 sec.
- F_2 - IRT 5 - 10 sec.
- F_3 - IRT 10 - 15 sec.
- F_4 - IRT 15 - 20 sec.
- F_5 - IRT 20 - 25 sec.
- F_6 - IRT 25 - 30 sec.
- F_7 - IRT 30 - 35 sec.
- F_8 - IRT 35 - 40 sec.

Appendix G

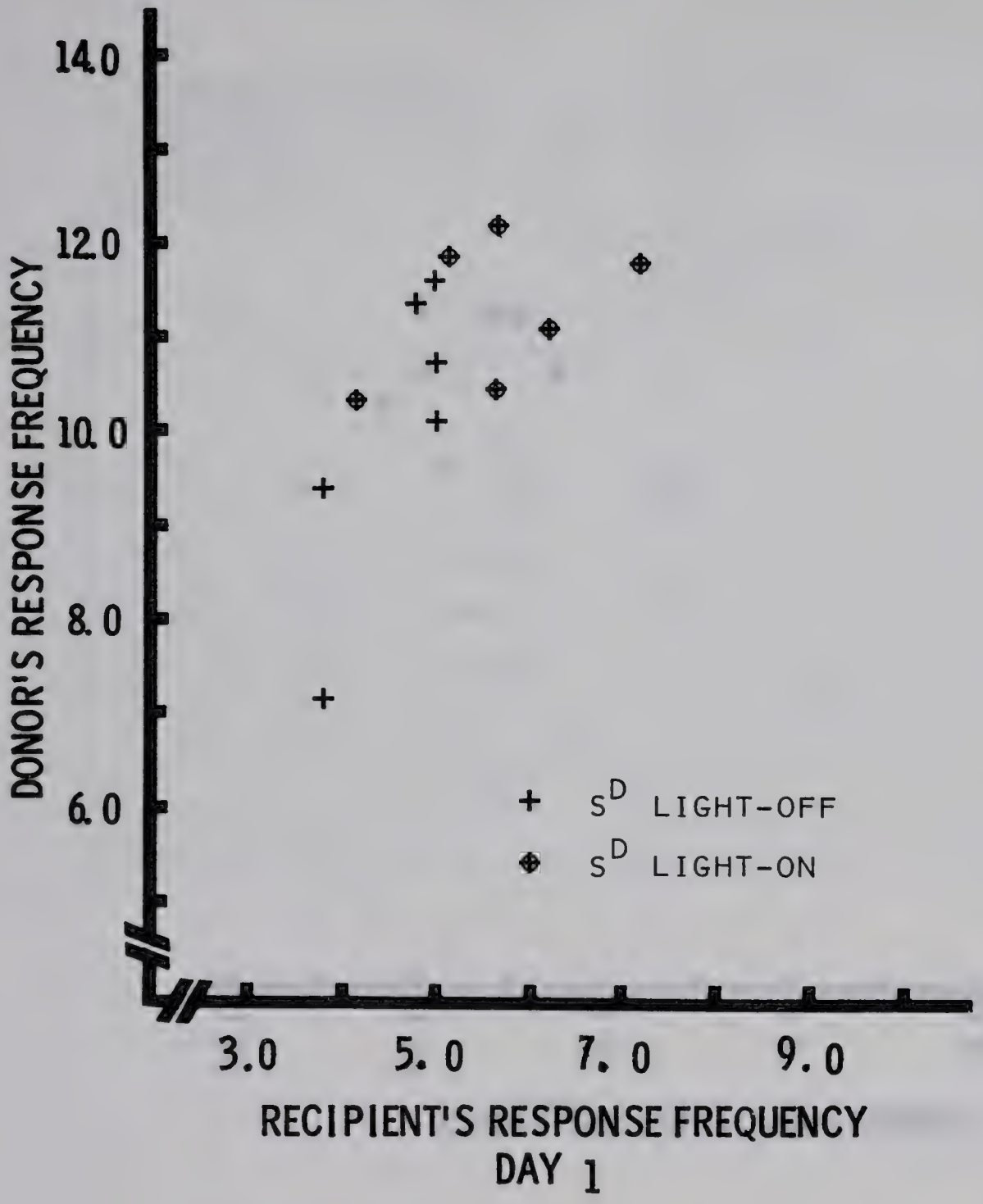
Intercorrelations of Donor's (Day 4) and Their Recipient's (Days 1 - 4) IRT Frequencies--
Same Stimulus Conditions -- Experiment III

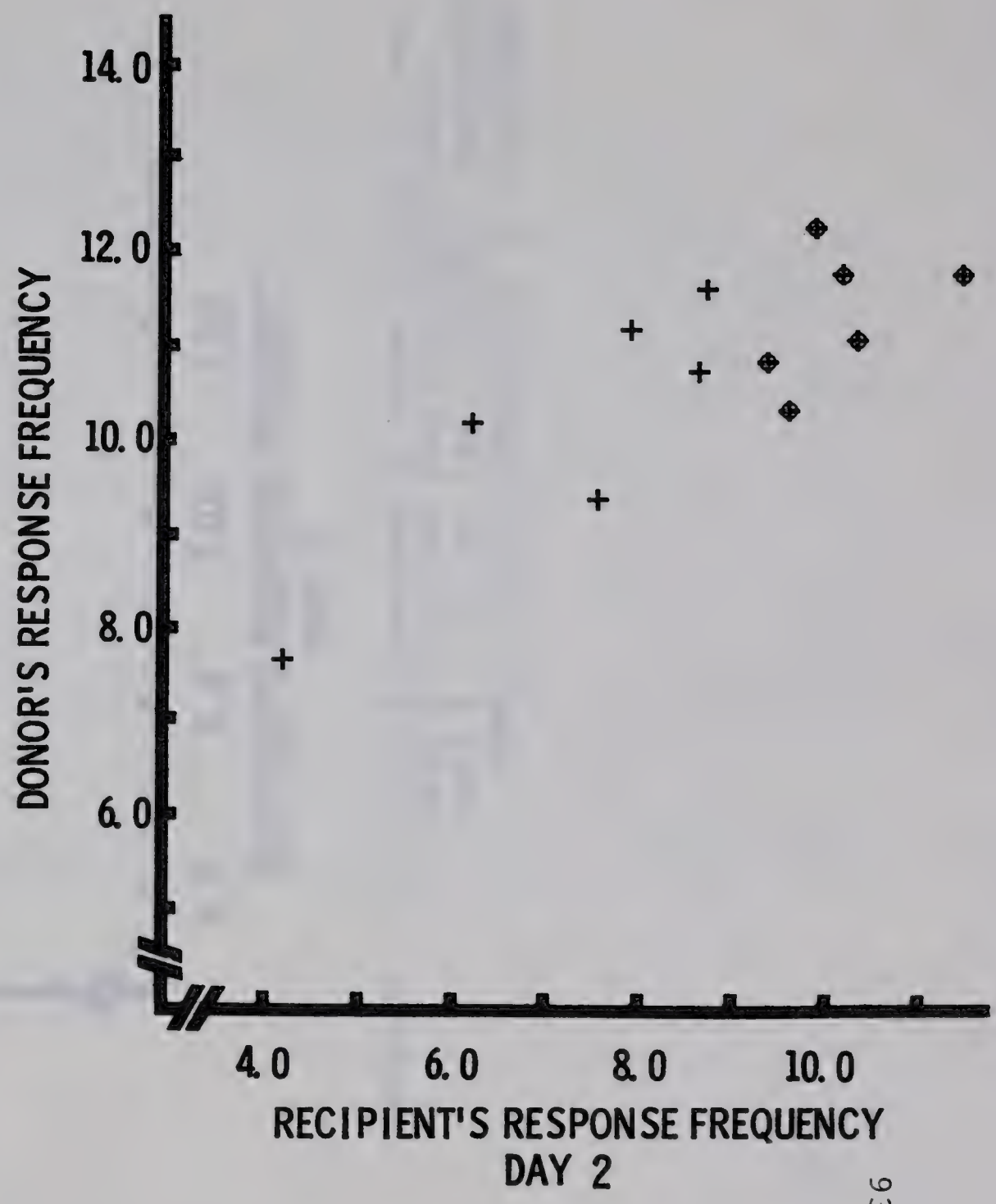
Day	Treatment	Recipients' IRTs: 0 - 5	-10	-15	-20	-25	-30	-35	-40 Sec.
1	Positive	+.096	+.061	-.526	-.558	+.688	-.287	+.183	+.359
	Negative	+.137	-.368	+.106	-.018	+.006	-.186	-.443	-.004
2	Positive	+.485	-.087	+.101	+.057	+.822	+.461	+.504	+.575
	Negative	+.072	+.246	-.094	-.169	+.011	+.137	-.849	-.155
3	Positive	+.326	-.089	+.366	+.404	+.591	+.365	+.339	+.046
	Negative	-.208	+.429	+.158	-.018	-.072	-.008	-.572	-.544
4	Positive	+.260	-.173	+.298	+.361	+.344	+.271	-.353	-.238
	Negative	+.390	+.055	+.060	-.279	-.388	-.273	-.326	+.245

Appendix H

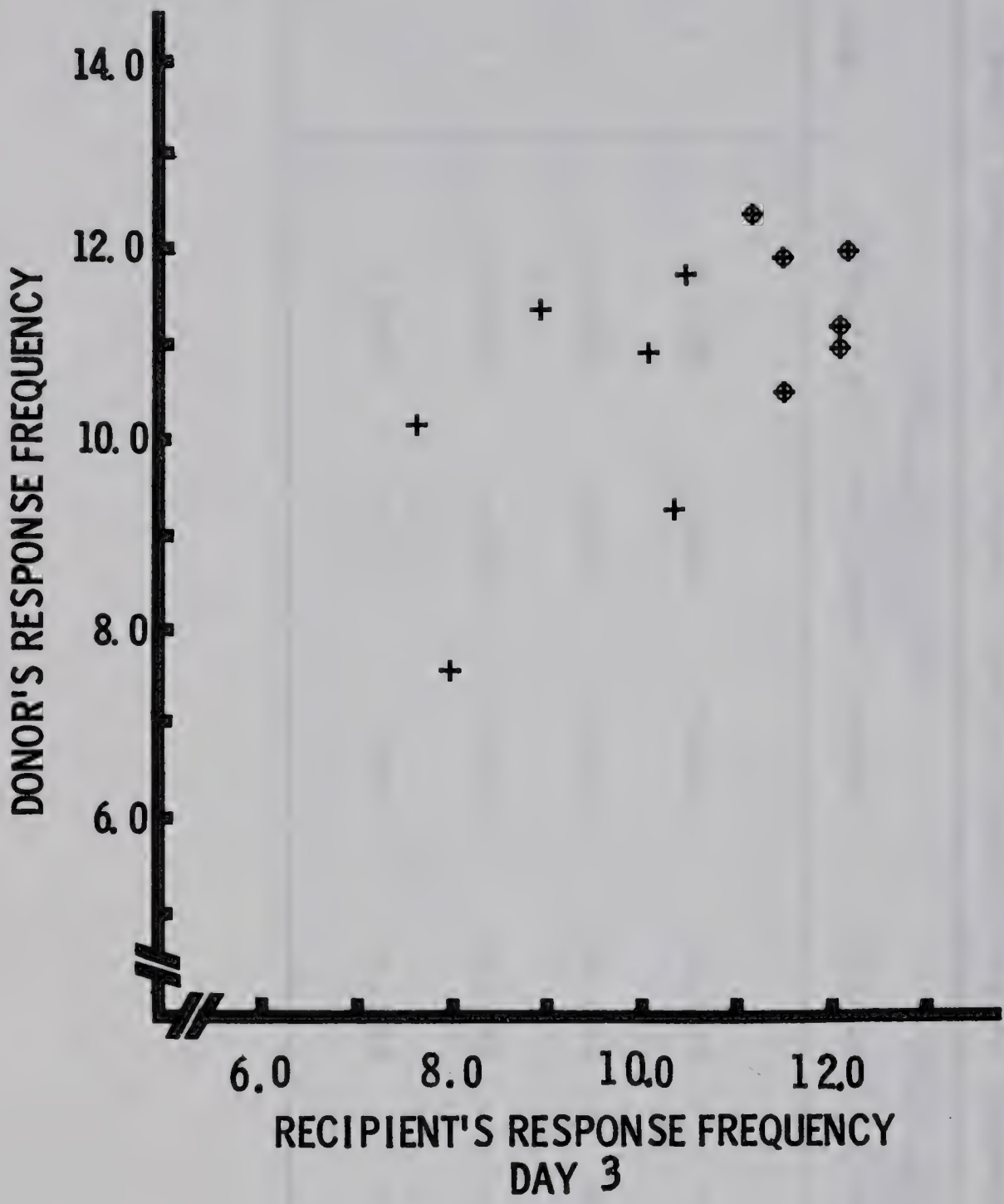
Intercorrelations of Donor's (Day 4) and Their Recipient's (Days 1 - 4) IRT Frequencies--
Opposite Stimulus Conditions -- Experiment III

Day	Treatment	Recipients' IRTs: 0 - 5	-10	-15	-20	-25	-30	-35	-40 Sec.
1	Positive	-.083	+.368	+.030	-.544	-.178	+.366	+.171	+.030
	Negative	-.388	-.007	-.039	+.038	-.068	+.448	+.331	-.278
2	Positive	-.562	+.432	+.054	+.007	-.331	+.594	+.273	+.431
	Negative	-.004	+.154	-.269	-.275	-.189	+.327	+.025	+.049
3	Positive	-.282	+.536	+.091	+.001	-.334	-.011	+.355	+.290
	Negative	+.148	+.251	-.240	+.071	-.053	+.342	+.319	+.237
4	Positive	-.370	+.356	+.093	-.113	-.420	+.192	+.083	+.461
	Negative	-.076	-.053	-.028	+.285	+.158	+.369	+.277	-.440





APPENDIX L1 AND L2 SCATTER DIAGRAMS OF DONOR'S (DAY 4) AND THEIR RESPECTIVE POSITIVE RECIPIENT'S (DAYS 1 - 3) IRT (20 - 25 SEC.) FREQUENCIES -- SAME STIMULUS CONDITIONS -- EXPERIMENT III



APPENDIX I.3 SCATTER DIAGRAMS OF DONOR'S (DAY 4) AND THEIR RESPECTIVE POSITIVE RECIPIENT'S (DAYS 1 - 3) IRT (20 - 25 SEC.) FREQUENCIES -- SAME STIMULUS CONDITIONS -- EXPERIMENT III

Appendix J

Original and Adjusted Intercorrelations of Donor's (Day 4) and Their Respective Positive Recipient's (Days 1 - 4) IRT (0 - 5 and 20 - 25 Sec.) Frequencies -- Same Stimulus Conditions -- Experiment III

Days	IRT: 0 - 5 sec. Stimulus Conditions			IRT: 20 - 25 sec. Stimulus Conditions		
	Original		Boxes	Original		Boxes
1	+.096	+.043	+.116	+.688	+.561	+.605
2	+.485	+.470	+.511	+.822	+.633	+.916
3	+.326	+.282	+.422	+.591	+.365	+.728
4	+.260	+.177	+.369	+.344	+.007	+.547

Appendix K

Days x IRTs Interaction for the Two Control Donor Groups -- Experiment III

	IRT:	0 - 5	-10	-15	-20	-25	-30	-35	-40	Sec. $\overline{IRTs}$
Day 1	Noninjected	12.677	6.334	5.970	5.094	4.700	3.596	2.439	1.632	5.305
	Saline	14.682	7.229	6.423	5.157	5.084	3.781	2.583	1.850	5.849
Day 2	Noninjected	5.350	3.872	5.063	5.196	8.271	4.290	2.357	1.321	4.465
	Saline	6.180	4.891	5.106	5.154	8.473	4.515	2.080	1.587	4.749
Day 3	Noninjected	3.699	3.121	3.858	4.178	10.269	3.409	1.704	1.294	3.942
	Saline	4.214	3.413	3.949	4.765	10.104	3.352	1.693	1.351	4.105
Day 4	Noninjected	2.777	2.574	3.301	3.412	10.937	3.039	1.549	1.302	3.612
	Saline	3.645	3.095	3.612	4.061	10.733	2.908	1.632	1.240	3.866
$\overline{Days}$	Noninjected	6.126	3.975	4.548	4.470	8.544	3.584	2.012	1.387	4.331
	Saline	7.180	4.657	4.773	4.784	8.599	3.639	1.997	1.507	4.642

Appendix L

Summary of the Analysis of Covariance Upon the Frequency of 0 - 5 Sec. IRTs -- Control Donor Groups -- Experiment III

Source of Variance	Sum of Squares	d.f.	Mean Square	F
Treatments (A)	27.19	1	27.19	
Stimulus Conditions (B)	9.75	1	9.75	
A x B	0.28	1	0.28	
Error: Pooled Subjects(C)	185.08	19	9.74	
Days (D)	1662.55	3	554.18	269.80*
D x A	7.68	3	2.56	
D x B	5.25	3	1.75	
D x A x B	4.76	3	1.59	
Error: D x C	121.19	59	2.05	
Total	2023.73	93		

*P<.005

Appendix M 1 and 2

Mean Response Frequencies for the Treatments x IRTs x Days Interaction (Analysis of Variance) -- Experimental Recipient Groups -- Experment III

		Day 1								
Treatment	IRT:	0 - 5	-10	-15	-20	-25	-30	-35	-40 Sec.	$\overline{IRTs}$
Positive		12.725	7.093	5.913	5.579	5.150	3.709	2.166	2.008	5.543
Neutral		13.635	6.260	5.794	5.094	4.820	3.612	2.358	1.746	5.415
Negative		15.570	7.445	6.323	5.431	5.394	3.752	2.030	1.603	5.944
$\overline{Treatments}$		13.977	6.933	6.010	5.368	5.121	3.691	2.185	1.785	5.634

		Day 2								
Treatment	IRT:	0 - 5	-10	-15	-20	-25	-30	-35	-40 Sec.	$\overline{IRTs}$
Positive		6.301	4.438	5.438	5.451	8.749	3.578	1.708	1.328	4.624
Neutral		6.236	4.538	5.277	5.242	8.596	4.005	1.793	1.400	4.636
Negative		5.939	5.102	5.260	4.723	9.038	4.024	1.838	1.302	4.653
$\overline{Treatments}$		6.159	4.692	5.325	5.139	8.795	3.869	1.779	1.343	4.638

Appendix $M_{3 \text{ and } 4}$

Mean Response Frequencies for the Treatments x IRTs x Days Interaction (Analysis of Variance) -- Experimental Recipient Groups -- Experiment III

		Day 3								
Treatment	IRT:	0 - 5	-10	-15	-20	-25	-30	-35	$-40_{Sec.}$	$\overline{IRT}$s
Positive		4.079	3.006	4.247	4.543	10.577	2.756	1.513	1.164	3.986
Neutral		3.884	2.878	3.490	4.424	10.312	3.548	1.720	1.201	3.932
Negative		4.720	3.640	4.081	3.948	10.669	2.905	1.677	1.290	4.116
$\overline{Treatments}$		4.227	3.175	3.939	4.305	10.519	3.069	1.637	1.219	4.011

		Day 4								
Treatment	IRT:	0 - 5	-10	-15	-20	-25	-30	-35	$-40_{Sec.}$	$\overline{IRTs}$
Positive		3.095	2.816	3.141	4.022	11.147	2.499	1.468	1.207	3.674
Neutral		3.028	2.593	3.356	4.292	11.120	2.587	1.345	1.103	3.678
Negative		4.268	3.028	4.159	4.017	11.067	2.557	1.286	1.225	3.951
$\overline{Treatments}$		3.464	2.812	3.552	4.110	11.111	2.547	1.367	1.179	3.768

Appendix N

Mean Response Frequency Differences Between the Neutral Group and the Positive and Negative Groups for the Treatments x IRTs x Days Interaction -- Experimental Recipient Groups -- Experiment III

Day	Treatment	IRT:	0 - 5	-10	-15	-20	-25	-30	-35	-40 Sec.
1	Positive		-.910	+.833	+.119	+.485	+.330	+.097	-.192	+.262
	Negative		+1.935	+1.185	+.529	+.337	+.574	+.140	-.328	-.143
2	Positive		+.065	-.100	+.161	+.209	+.153	-.427	-.085	-.072
	Negative		-.297	+.564	-.017	-.519	+.442	+.019	+.045	-.098
3	Positive		+.205	+.128	+.757	+.119	+.265	-.792	-.207	-.037
	Negative		+.836	+.762	+.591	-.476	+.357	-.643	-.043	+.089
4	Positive		+.067	+.223	-.214	-.270	+.027	-.088	+.123	+.104
	Negative		+1.240	+.435	+.803	-.275	-.053	-.030	-.059	+.122
$\overline{\text{Days}}$	Positive		-.146	+.271	+.206	+.136	+.194	-.303	-.090	+.064
	Negative		+.938	+.737	+.477	-.260	+.330	-.131	-.096	-.008

Appendix O

Mean Response Frequencies for the Treatments x IRT Interaction -- Experimental Recipient Groups -- Experiment III

		Days All							
Treatment	IRT:	0 - 5	-10	-15	-20	-25	-30	-35	-40 Sec.	$\overline{IRTs}$
Positive		6.550	4.338	4.685	4.899	8.906	3.135	1.714	1.427	4.457
Neutral		6.696	4.067	4.479	4.763	8.712	3.438	1.804	1.363	4.415
Negative		7.624	4.804	4.956	4.503	9.042	3.309	1.708	1.355	4.666
$\overline{Treatments}$		6.957	4.403	4.707	4.730	8.887	3.294	1.742	1.382	4.513

Appendix P

Untransformed Mean Response Frequencies for the Treatments x IRTs (0 - 20 Sec. and 20 - 40 Sec.) for Days 1 - 4 -- Experimental Recipient Groups -- Experiment III

Day	Treatment	S^{Δ} (IRTs 0 - 20 sec.)	S^{D} (IRTs 20 - 40 sec.)
	Positive (N = 12)	280.4	48.6
1	Neutral (N = 12)	288.6	43.5
	Negative (N = 12)	375.5	46.8
	Positive (N = 12)	124.2	94.7
2	Neutral (N = 12)	117.9	95.9
	Negative (N = 12)	113.2	101.1
	Positive (N = 12)	71.0	122.5
3	Neutral (N = 12)	56.8	122.9
	Negative (N = 12)	72.4	123.8
	Positive (N = 12)	46.8	132.8
4	Neutral (N = 12)	46.8	131.2
	Negative (N = 12)	61.1	129.3

Appendix Q

Adjusted Mean Response Frequencies of the Analysis of Covariance Treatments x Days Interaction for IRTs of 0 - 5 Seconds -- Experimental Recipient Groups -- Experiment III

Treatment	Day 1	Day 2	Day 3	Day 4
Neutral	13.75	6.35	4.00	3.14
Positive	12.55	6.13	3.90	2.92
Negative	15.63	6.00	4.78	4.33

Appendix $R_{1 \text{ and } 2}$

Mean Response Frequencies of the Analysis of Variance Stimulus Conditions x IRTs x Days Interaction -- Control Donor Groups -- Experiment III

Stimulus Conditions	IRT:	0 - 5	-10	-15	-20	-25	-30	-35	-40	$\overline{\text{IRTs}}$ Sec.
					Day 1					
S^D-Light-on		12.976	6.909	6.298	5.028	5.250	3.728	2.414	1.771	5.547
S^D-Light-off		14.382	6.654	6.094	5.223	4.534	3.650	2.607	1.711	5.607
$\overline{\text{Stimulus Conditions}}$		13.679	6.781	6.196	5.125	4.892	3.689	2.511	1.741	5.577
					Day 2					
S^D-Light-on		5.649	4.721	4.981	4.843	9.230	4.028	2.152	1.268	4.609
S^D-Light-off		5.880	4.043	5.188	5.507	7.514	4.777	2.286	1.640	4.604
$\overline{\text{Stimulus Conditions}}$		5.765	4.382	5.082	5.175	8.372	4.402	2.219	1.454	4.607

Appendix R_{3} and 4

Means Response Frequencies of the Analysis of Variance Stimulus Conditions x IRTs x Days Interaction -- Control Donor Groups -- Experiment III

		Day 3								
Stimulus Condition	IRT:	0 - 5	-10	-15	-20	-25	-30	-35	-40 Sec.	$\overline{\text{IRTs}}$
S^{D}-Light-on		3.670	3.532	3.546	4.045	10.804	2.990	1.495	1.233	3.914
S^{D}-Light-off		4.243	3.002	4.261	4.898	9.568	3.771	1.903	1.412	4.132
$\overline{\text{Stimulus Conditions}}$		3.956	3.267	3.903	4.472	10.186	3.380	1.699	1.322	4.023

		Day 4								
Stimulus Condition	IRT:	0 - 5	-10	-15	-20	-25	-30	-35	-40 Sec.	$\overline{\text{IRTs}}$
S^{D}-Light-on		3.062	2.956	3.079	3.082	11.311	2.701	1.472	1.248	3.614
S^{D}-Light-off		3.360	2.712	3.834	4.392	10.359	3.246	1.710	1.294	3.863
$\overline{\text{Stimulus Conditions}}$		3.211	2.834	3.456	3.737	10.835	2.974	1.591	1.271	3.739

Appendix $S_{1 \text{ and } 2}$

Mean Response Frequencies of the Stimulus Conditions x IRTs x Days Interaction -- Experimental Recipient Groups -- Experiment III

Stimulus Condition	IRT: 0 - 5	-10	-15	-20	-25	-30	-35	-40 Sec.	$\overline{\text{IRTs}}$
				Day 1					
S^D-Light-on	14.600	6.968	6.029	5.579	5.543	3.882	2.164	1.676	5.805
S^D-Light-off	13.353	6.897	5.992	5.157	4.699	3.501	2.205	1.895	5.462
$\overline{\text{Stimulus Conditions}}$	13.977	6.933	6.010	5.368	5.121	3.691	2.185	1.785	5.634

Stimulus Condition	IRT; 0 - 5	-10	-15	-20	-25	-30	-35	-40 Sec.	$\overline{\text{IRTs}}$
				Day 2					
S^D-Light-on	5.440	4.348	4.748	4.487	9.921	3.647	1.524	1.196	4.414
S^D-Light-off	6.877	5.036	5.902	5.790	7.668	4.091	2.035	1.491	4.861
$\overline{\text{Stimulus Conditions}}$	6.159	4.692	5.325	5.139	8.795	3.869	1.779	1.343	4.638

Appendix $S_{3 \text{ and } 4}$

Mean Response Frequencies of the Stimulus Conditions x IRTs x Days Interaction -- Experimental Recipient Groups -- Experiment III

		Day 3								
Stimulus Condition	IRT:	0 - 5	-10	-15	-20	-25	-30	-35	-40 Sec.	$\overline{\text{IRTs}}$
S^D-Light-on		3.430	2.825	3.024	3.348	11.407	2.684	1.428	1.234	3.673
S^D-Light-off		5.024	3.524	4.855	5.262	9.631	3.454	1.845	1.203	4.350
$\overline{\text{Stimulus Conditions}}$		4.227	3.175	3.939	4.305	10.519	3.069	1.637	1.219	4.011

		Day 4								
Stimulus Condition	IRT:	0 - 5	-10	-15	-20	-25	-30	-35	-40 Sec.	$\overline{\text{IRTs}}$
S^D-Light-on		2.817	2.381	2.880	3.458	11.666	2.306	1.304	1.087	3.487
S^D-Light-off		4.110	3.243	4.224	4.763	10.556	2.789	1.429	1.271	4.048
$\overline{\text{Stimulus Conditions}}$		3.464	2.812	3.552	4.110	11.111	2.547	1.367	1.179	3.768

Appendix T

Summary of the Analysis of Covariance Upon the Latency Indices -- Control Donor Groups -- Experiment III

Source of Variance	Sum of Squares	d.f.	Mean Square	F
Treatments (A)	0.000	1	0.000	
Stimulus Conditions (B)	0.243	1	0.243	6.40*
A x B	0.009	1	0.009	
Error: Pooled Subjects(C)	0.722	19	0.038	
Days (D)	1.188	3	0.396	56.31*
D x A	0.014	3	0.000	
D x B	0.037	3	0.012	
D x A x B	0.002	3	0.001	
Error: D x C	0.415	59	0.007	
Total	2.630	93		

*P<.005

Appendix U

Summary of the Analysis of Covariance Upon the Latency Indices -- Experimental Recipient Groups -- Experiment III

Source of Variance	Sum of Squares	d.f.	Mean Square	F
Treatments (A)	0.032	2	0.016	
Stimulus Conditions (B)	0.326	1	0.326	15.64*
A x B	0.049	2	0.024	
Error: Pooled Subjects(C)	0.604	29	0.021	
Days (D)	2.385	3	0.795	140.93*
D x A	0.032	6	0.005	
D x B	0.039	3	0.013	
D x A x B	0.020	6	0.003	
Error: D x C	0.502	89	0.006	
Total	3.989	141		

*P<.005

Appendix U

Summary of the Analysis of Covariance Upon the Latency Indices -- Experimental Recipient Groups -- Experiment III

Source of Variance	Sum of Squares	d.f.	Mean Square	F
Treatments (A)	0.032	2	0.016	
Stimulus Conditions (B)	0.326	1	0.326	15.64*
A x B	0.049	2	0.024	
Error: Pooled Subjects(C)	0.604	29	0.021	
Days (D)	2.385	3	0.795	140.93*
D x A	0.032	6	0.005	
D x B	0.039	3	0.013	
D x A x B	0.020	6	0.003	
Error: D x C	0.502	89	0.006	
Total	3.989	141		

*p<.005